1 Discussion

Drosophila (or the fruit fly) is a well-established animal model for studying sleep due to its striking similarities with vertebrates in sleep regulation (Shafer and Keene 2021; Andreani et al. 2022). R5 neurons have been reported to play an important role in sleep regulation in the fruit flies. Specifically, these neurons are thought to encode the homeostatic sleep drive in these animals (Sha Liu et al. 2016). Similar to mammals, Slow-Wave Activity (SWA) has been observed in *Drosophila* brain during sleep and following sleep deprivation, and is believed to be generated at the level of the R5 neurons (Raccuglia et al. 2019). Furthermore, these neurons exhibit tonic spiking activity during the daytime, and bursting at night and after sleep deprivation (Suárez-Grimalt and Raccuglia 2021; Sha Liu et al. 2016).

Despite these observations, the cellular mechanisms underlying R5 neuronal activity remain poorly understood. Gaining insight into these mechanisms is crucial for a better understanding of the cellular basis of sleep regulation in *Drosophila*. In this study, conductance-based computational models were used to investigate the possible mechanisms responsible for several experimental findings related to the R5 activity.

First, electrophysiological studies in R5 neurons of *Drosophila* have reported that their activity switches from tonic firing during the daytime to bursting during sleep and following sleep deprivation (Sha Liu et al. 2016; Suárez-Grimalt and Raccuglia 2021). A study on gene expression in *Drosophila* identified a negative correlation between increased sleep drive and the expression of a gene encoding a potassium channel known as ether-à-go-go (EAG) (Dopp et al. 2024). The current thesis demonstrated that variation in the expression of the EAG channel could be a biologically plausible mechanism underlying the transition of R5 activity from bursting to tonic spiking, where the latter results from the reduction in the number of spikes per burst.

Second, an unpublished study on Drosophila R5 neurons by Anatoli Ender and David Owald reported the existence of slow oscillations (with the period on the order of a few seconds) following the blockade of Na^+ channels. These slow oscillations were hypothesized to be calcium spikes mediated by the T-type Ca^{2+} channels. However, the results of the current study indicate that Ca^{2+} channels alone cannot explain the observed slow-frequency (< 1 Hz) oscillations due

to their relatively fast kinetics. Therefore, other, or additional, mechanisms should be involved to account for the large interspike intervals.

Third, another unpublished study of Anatoli Ender, and David Owald showed that knockdown of the T-type calcium channels in R5 neurons causes an increase in the minimal recorded membrane potential during bursting activity. This observation is unintuitive at first glance, as calcium is a depolarizing current and removing it is expected to have an opposite (i.e. hyperpolarizing) effect. Simulations showed that Ca^{2+}-activated K^{+} channels could be the underlying mechanism behind this observation. It was demonstrated that these channels can explain experimental observations and cause an increase in the minimal resting membrane potential following blockade of the T-type calcium channels.

1.1 Bursting and transition to tonic spiking

It has been shown that compound SWA in *Drosophila* R5 neurons results from the synchronized activity of single R5 neurons (Raccuglia et al. 2019). SWA itself has been associated with sleep need, quality and sensory gating during sleep (Suárez-Grimalt and Raccuglia 2021; Raccuglia et al. 2019; Gent et al. 2018). R5 neurons start bursting and synchronize their activity with increasing sleep need (Suárez-Grimalt and Raccuglia 2021). It has been reported that bursting can enhance the reliability of signal transmission by overcoming synaptic transmission failures, induce spikes more reliably in postsynaptic neurons, and influence synaptic plasticity more effectively in comparison to single spikes (Lisman 1997; Kim and Lim 2019). Thus, the transition between spiking and bursting could potentially facilitate synchronization of R5 neurons and, consequently, generation of the compound SWA in *Drosophila* central complex. However, the cellular mechanisms underlying this change in firing pattern are not yet fully understood.

Transition between tonic spiking and bursting has been studied in various animal models, including mammalian thalamocortical neurons (X.-J. Wang 1994; Suzuki and Rogawski 1989; Vickstrom et al. 2020; Shaolin Liu and Shipley 2008; McCormick and Huguenard 1992). Different mechanisms have been proposed to achieve both types of activity in the same cell.

The early work by Wang (X.-J. Wang 1994) proposed a model that exhibits bursting behaviour mediated by Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) and T-type channels (see "T-type and HCN channels" in Section **??** for detailed description of correspond-

ing bursting mechanism). In short, the HCN and T-type channels form the slow subsystem, that first drives the membrane potential to the spiking threshold, and then to a hyperpolarized state. The strength of this drive depends on the magnitude of the hyperpolarizing external current: the stronger the hyperpolarizing current, the more HCN channels activate and more T-type channels deinactivate (i.e. their inactivation gate opens). Apart from bursting, the model exhibits tonic spiking in two regimes. The first occurs when the hyperpolarizing current is too weak to fully activate the slow subsystem, resulting in a single spike. The Second occurs when the external current is depolarizing. In this case, the membrane potential is directly driven to the spiking threshold, resulting in tonic spiking at a frequency comparable to that within bursts.

However, the model proposed by Wang exhibits large variations in bursting frequency in response to small changes in the input current (see also Figure **??**). Furthermore, the frequency of the tonic firing in R5 neurons is relatively low compared to the frequency of spikes within bursts (Figures **??** and **??**). Thus, the transition between tonic spiking and bursting in R5 neurons is less likely to be mediated by external input.

Bursting is defined as a slow alternating transition between spiking and steady states (Rinzel 1987), while tonic spiking refers to the periodic firing of single spikes. By definition, if the number of spikes per burst is reduced to one, the activity becomes indistinguishable from tonic spiking. Thus, a change in the excitability of R5 neurons between day and night may result in a reduction in the number of spikes per burst, underlying the observed shift in the activity patterns.

Several mechanisms have been proposed that reduce the number of spikes per burst, including upregulation of the A-type potassium channels, and downregulation of T- or L-type calcium channels (Franci, Drion, and Sepulchre 2018; Park, Rubchinsky, and Ahn 2021). Since potassium currents are hyperpolarizing, upregulation of the underlying channels opposes depolarization and can promote burst termination. Conversely, calcium currents are depolarizing, and downregulation of calcium channels may limit the ability to sustain bursting.

A recent study reported that expression of the gene encoding the potassium EAG channel negatively correlated with the increasing sleep drive (Dopp et al. 2024) in *Drosophila* R5 neurons. Since R5 neuronal activity increases with elevated sleep drive (Sha Liu et al. 2016), upregulation of the EAG channel in rested flies may contribute to early burst termination. Thus, the observed tonic spiking may result from a reduced number of spikes per burst, rather than

from a fundamental change in the intrinsic response properties of R5 neurons.

Indeed, simulations showed that increasing the maximal conductance of the EAG channel can induce a transition from bursting to tonic spiking by the above-described mechanism. Bifurcation analysis revealed that this transition is mediated by the modulation of the V-nullcline in the fast (i.e. spiking) subsystem. Specifically, increasing the maximal conductance of the EAG channel alters the geometry of the V-nullcline such that the system spends less time in the spiking state - the region of the phase space where a stable limit cycle exists (Figure **??**).

To investigate whether upregulation of the EAG channel could induce transition from bursting to tonic spiking, the parameters of the EAG channel were chosen such that its current does not significantly affect the bursting period. In the Wang model, this required a steep steady-state activation curve for the EAG channel, as the model exhibits large variations in bursting period with small changes in the external current (see also Figure **??**). If the EAG channel is not fully deactivated during the interspike interval, it could effectively introduce an additional depolarizing current to the original Wang model (i.e., the model without the EAG channel) during the interspike interval, thus oppose depolarizing currents and, consequently, reduce the oscillation frequency.

The above-mentioned requirement not to modulate the oscillation period is a strict requirement with regard to the R5 neurons. As illustrated in Figure **??**, the frequency power spectra of R5 neurons have peaks at lower frequencies than 1 Hz (bursting frequency at night). Thus, this shift could potentially also be attributed to the upregulation of EAG channels during the daytime, in addition to variations in synaptic input from other neurons.

1.2 What type of bursting behavior do R5 neurons exhibit?

To my knowledge, the cellular mechanisms underlying bursting in R5 neurons have not been investigated. It remains unclear whether these neurons burst due to their intrinsic properties or if their bursting is driven by oscillatory input. Electrophysiological studies have suggested that slow-wave activity SWA may be generated at the level of R5 neurons (Raccuglia et al. 2019), which supports the hypothesis that their bursting arises intrinsically rather than being evoked by periodic external input.

As discussed in Section **??**, neurons that exhibit bursting behaviour due to their intrinsic

properties can be classified based on their response characteristics to the external input, and based on the cellular mechanisms that initiate and terminate bursts.

It has been shown that even for a fixed model structure (i.e. fixed set of ion channels, ion dynamics, etc.) variations in the maximal conductance of a single ion channel can lead to qualitatively different dynamical behaviours, even when the resulting voltage traces appear similar under baseline condition (i.e. prior to parameter variation) (Alonso and Marder 2019). Thus, multiple mechanisms could the observed firing pattern in R5 neurons.

1.3 Slow-freqeucny oscillations following Na^+ channel blockade

The slow-frequency spiking with a period of several seconds has been observed following Na^+ channel blockade and is hypothesized to be mediated by T-type calcium channels. Although these channels may be responsible for generating the spikes themselves, the long interspike intervals cannot be fully explained by the T-type current alone, given that the gating kinetics of these channels operate on a timescale of approximately 100 ms (Jeong et al. 2015).

A potential mechanism underlying the slow interspike interval could involve the activation of a depolarizing current with slow kinetics, which gradually drives the membrane potential toward the spiking threshold. Alternatively, it could arise from a slowly deactivating hyperpolarizing current, which delays the dominance of depolarizing currents and postpones spike initiation.

Regardless of the current type (hyperpolarizing, or depolarizing) and gating type (inactivating or non-inactivating), the gating variables responsible for the slow interspike interval should evolve on a timescale of approximately one second at membrane potentials observed during the interspike interval. However, their kinetics must be significantly faster at more depolarized and/or hyperpolarized potentials in order to allow fast recovery during the brief duration of a spike ($\sim$100 ms, Figure **??**).

In the context of the first-order kinetic gating models (when activation and inactivation gates have only two - open and closed states), this implies a steep voltage dependence of the gating time constant $\tau(V)$ (see Equation **??**). Specifically, it must vary by an order of magnitude within a relatively narrow voltage range (15-20 mV), corresponding to the difference between the membrane potentials at the spike peak and at steady state (Figure **??**).

Alternatively, the kinetics may be modelled using higher-order kinetic schemes. These mod-

els account for gating mechanisms involving more than two states, including multiple closed or inactivated states and distinct transition pathways between them. Such models are commonly implemented using Markov chains (Mangold et al. 2021). For example, the model proposed by X. J. Wang, Rinzel, and Rogawski (1991) for the T-type calcium channel includes one open and two closed states in the inactivation gate of the channel, thereby capturing more complex gating dynamics than a first-order kinetic model.

Another mechanism, that to my knowledge has not been reported in the literature, may involve the interplay between calcium- and voltage-gated potassium channels (such as the large-conductance BK channel), voltage-gated calcium channels (e.g., T- or L-type), and intracellular calcium dynamics. Similar to the previously described mechanism, slow deactivation of potassium channels could lead to a gradual dominance of depolarizing currents, eventually driving the membrane potential toward the spiking threshold.

Which mechanisms might underlie the prolonged deactivation of the BK channels? Full activation of BK channels requires both - an increase in intracellular calcium concentration and sufficient membrane depolarization (Shaolin Liu and Shipley 2008). While many modelling studies assume rapid calcium decay, typically ranging between 1-13 ms (Huguenard and McCormick 1992; Golomb, Yue, and Yaari 2006; Park, Rubchinsky, and Ahn 2021), experimental studies have reported two distinct timescales of calcium decay, a fast (10ms) and with a slow component on the order of hundreds of milliseconds (Theis et al. 2016; Koester and Sakmann 2000).

Moreover, calcium channels may continue to mediate inward currents at membrane potentials observed during the interspike interval. Taken all together, I suggest the following scenario: the initial rapid hyperpolarization during a spike could be mediated by BK channels, whose activity is supported by both depolarization and the rise in intracellular calcium following the spike onset. At hyperpolarized potentials, BK channels begin to inactivate due to voltage sensitivity and/or the fast component of the calcium decay. However, if the activation gate of BK channels remains partially open near the resting potential, they could undergo slow deactivation driven by the slow component of calcium decay.

Combining these altogether, if the initial fast hyperpolarization could be mediated by the BK channels, the first, fast decay in the membrane potential during spike can be mediated by the BK

channels that inactivate due to their voltage dependence and the fast component of the calcium decay. However, if the activation gate of these channels is open in the membrane potentials where the neuron exhibits almost steady state, they can be slowly deactivated by the slow component of the calcium removal. This deactivation might be further slowed down by the current mediated by the calcium channels. Thus, a combination of the slow timescale of the calcium decay, together with calcium influx during steady state, could explain the large oscillation periods between the observed spikes.

This deactivation may be prolonged by continuous calcium influx through T- or L-type calcium channels active at subthreshold potentials. Thus, continuous calcium entry, together with deactivation of BK channels via the slow component of calcium decay, could explain the long interspike intervals in the R5 neurons following blockade of sodium channels.

Lastly, the presence of oscillations after blockade of sodium channels does not exclude the possibility that the neuron receives input from other sources, such as electrical coupling via gap junctions, or interactions with astrocytes, rather than through conventional synaptic transmission.

The mechanism responsible for generating slow oscillations in R5 neurons following sodium channel blockade, as well as the potential involvement of external sources, remains unknown. Further experimental studies are needed to investigate this observation.

1.4 Increase in minimal resting membrane potential following T-type channel knockdown

This finding is particularly interesting, as to my knowledge, such an effect of the T-type calcium channel knockdown on the minimum membrane potential has not been previously reported or modelled. In fact, the opposite effect has been demonstrated Alonso and Marder 2019. One modelling study demonstrated that, for specific parameter combinations, the model exhibited an increase in the minimum membrane potential following T-type channel blockade. However, this effect was reversed once more than approximately 20% of the channels were blocked. Notably, authors did not explicitly discuss this effect — it can only be seen in one of the figures.

In this work, it was demonstrated that calcium-activated potassium channels can underlie

this experimental observation. If the current through such ion channel is strong enough, then reducing calcium influx will result in reduced activation of these channels, and, thereby, reduced hyperpolarizing drive. The reduced hyperpolarizing drive, on the other hand, can cause the minimal membrane potential to go to more depolarized levels.

As the conductance-based models are high-dimensional, I am not aware of any method which could be used to fully describe the response properties of neurons to all parameter variations. Identification of such behaviour of the dynamical system requires careful examination of specific parameter regimes, and attention to specific details, such as minimal membrane potential. Thus, it is possible that other models than the Goldman may exhibit the above-described effect, but it might not have been explored due to a focus on other research questions.

The Goldman model exhibits burst termination due to calcium-activated potassium channels. Importantly, our result does not necessarily imply that bursting must be terminated via calcium-activated potassium channels. In principle, a similar effect on minimum membrane potential could be observed regardless of the underlying bursting mechanism. However, further investigation is necessary, and this question can be a direction for future research.

1.5 Limitations of current approach

Although the current work suggests involvement of specific ion channels to reproduce the observed R5 neuronal responses, there are several limitations to the approach.

First, the proposed mechanisms behind the experimental observation are single examples of how the experimental observation could be reproduced with a modelling approach. Other, more complex mechanisms may involve calcium-dependent modulation of synaptic strength or gene expression.

Second, the current study concentrated on single-compartment conductance based models. Such models assume uniform distribution of the ion channels across a neuron, as well as do not account for morphology. It has been shown that models that account for these fit experimental data better and can be more robust to parameter variations (Destexhe et al. 1998).

Although R5 neurons share similar functional characteristics with thalamic neurons, morphology of neurons in *Drosophila* is significantly different from those in vertebrates. Unlike vertebrates, *Drosophila* neurons have unipolar morphology. Because of this morphology, synap-

tic potentials traveling from dendrites to the spike initiation zone bypass the cell body. Because of this morphology, the cell body of *Drosophila* neurons is electronically segregated from other cell regions, suggesting that it is not involved in synaptic integration (Gouwens and Wilson 2009; Tuthill 2009). Furthermore, it has been found that, in contrast to vertebrates, dendrites of *Drosophila* neurons (specifically, Kenyon cell (KC)) are not solely postsynaptic, but also form presynaptic active zones (Christiansen et al. 2011). Thus, the single-compartment model might be too simple to fully explain experimental observations.

Furthermore, as described in Section **??**, the experimental studies found a reversible increase in cytosolic calcium concentration in R5 neurons in the morning, evening, and following sleep deprivation (andreaniCircadianProgrammingEllipsoid2022,liuSleepDriveEncoded2016). Increased calcium levels may be attributed to the increased calcium influx through T-type channels during night and sleep deprivation. However, as discussed in Section **??**, release of calcium from Ellipsoid Body (EB) might be an alternative mechanism contributing to the increase in intracellular calcium levels.

Although this work did not concentrate on the possible effects of diurnal variation of intracellular calcium concentration, this could be an additional mechanism that may modulate R5 activity. However, it still remains unclear if and to what extent increased level of calcium can affect the response properties of R5 neurons.

Furthermore, what is the mechanism behind burst initiation and termination in R5 neurons still remains unclear. It is hard to address this question by conductance-based models, as, even in the same model, many different parameter combinations may lead to the model exhibiting the same voltage characteristics Alonso and Marder 2019; Prinz, Billimoria, and Marder 2003.

Tuning the parameters of a conductance-based model to produce specific dynamical characteristics is a hard problem. First, due to large number of parameters, investigating every parameter combination even in the constrained parameter space is computationally inefficient. Second, predicting the effects of an arbitrary parameter perturbation on the solution is a challenging and largely unresolved problem (Alonso and Marder 2019). Several approaches have been proposed to find parameters reproducing the desired dynamics. Prinz and colleagues constructed a database of 1.7 million single-compartment neurons by individually varying 8 maximal conductances for one particular model of choice (Prinz, Billimoria, and Marder 2003). Others proposed

using evolutionary algorithms to find a set of parameter combinations leading to similar voltage responses in model neurons (Alonso and Marder 2019). It has been demonstrated that such multiple parameter combinations can result in qualitatively the same membrane potential dynamics, even within the same single-compartment model (Alonso and Marder 2019).

1.6 Summary and outlook

The current work showed that variation of the EAG channel in response to the increased sleep drive is a biologically plausible mechanism to switch R5 neuronal activity from tonic spiking during the day to bursting at night. However, experimental verification is necessary to verify this computational result.

Within this thesis, parameters of the EAG channel were hand-tuned to observe the switch between spiking to bursting without affecting the oscillation period. Although, as discussed above, this condition might not be necessary, what conditions should be met the EAG channels to exhibit such a modulatory effect should be investigated.

Furthermore, switching to bursting activity at night has been suggested to facilitate compound SWA in R5 neurons. If variation of EAG channel expression induces a swich from tonic firing to bursting, can one induce synchronization within R5 neurons by changing the maximal conductance of the EAG channels? This question is yet to be investigated.

As presented in Section **??**, oscillatory power in R5 neurons increases following sleep deprivation in comparison to sleep. If the upregulation of the EAG channel causes R5 neurons to synchronize, the increased power following sleep deprivation might be due to increased external drive from R5 presynaptic neurons.

Another experimental observation reported by Anatoli Ender and David Owald showed an increase in the minimal resting membrane potential following T-type calcium channel knockdown. To my knowledge, such an effect has never been studied. Current work demonstrated that calcium-activated potassium channels can be the possible mechanism underlying such an effect. Although this was demonstrated on the model, which exhibited burst termination through Ca^{2+}-activated K^+ channels, this mechanism could potentially work for other types of bursting neurons as well, regardless of the mechanism for burst termination. It would be interesting to test this hypothesis on different bursting neuronal models and investigate what are the possible

limitations of the approach.

The third experimental observation, which was addressed in this work, was slow oscillations in the membrane potential following sodium channel blockade. Contrary to the hypothesis, the current work showed that the slow component of the oscillations cannot be mediated solely by the T-type calcium channels. Although what mechanism that causes the spike onset is still unclear, the results suggest involvement of a slower mechanism than the kinetic time constants of the T-type channels. One such mechanism was suggested above. However, more experimental and modelling studies should be carried out to investigate underlying cellular processes that can cause these slow oscillations.

Although the work suggests several mechanisms that might govern responses of R5 neurons, more work should be done to construct a biologically plausible model for the R5 neuron that could exhibit all of the above-mentioned response features. Furthermore, the current study did not concentrate on modelling the details of R5 responses, such as, temporal width of the bursts, or the number of spikes per burst. Currently, there is a limited amount of experimental and modelling studies on cellular mechanisms behind R5 responses. Nevertheless, the present work represents a step towards bridging this gap.