1 Discussion

Drosophila is a well-established animal model for studying sleep due to its striking similarities in sleep regulation with vertebrates Shafer and Keene 2021; Andreani et al. 2022. R5 neurons have been reported to play important role in sleep regulation in the fruit flies. Specifically, these neurons are thought to encode the homeostatic sleep drive in these animals 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 bursing at night and after sleep deprivation Suárez-Grimalt and Raccuglia 2021; Liu et al. 2016.

Descpite these observations, the cellular mechanisms underlying R5 neuronal activity remains poorly understood. Gaining insight into these mechanisms is crucial for 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 bfrom tonic firing during the daytime to bursting during sleep and following sleep deprivation (Liu et al. 2016; Suárez-Grimalt and Raccuglia 2021). A study on gene expression in *Drosophila* identified a negative correlation between the expression of a gene encoding a potassium channel known as ether-à-go-go (EAG) and increased sleep drive Dopp et al. 2024. This study demonstrated that variation in the expression of EAG channel might be biologically plausible mechanism underlying transition of R5 activity from tonic spiking to bursting, by reducing the number of spikes per burst.

Second, an unpublished study by David Owald, Anatoli Ender, and colleagues reported the existance of slow oscillations on the order of few seconts following the blockde of Na^+ channels in Drosophila R5 neurons. These slow oscillations were hypothesized to be mediated by T-type Ca^{2+} channels. However, the results of the current study indicate that Ca^{2+} channels alone cannot explain such slow-frequency oscillations due to their relatively fast kinetics. Therefore, other, or additional mechanisms should be present to account for the observed large interspike

intervals.

Third, another unpublished study of David Owald, Anatoli Ender and colleagues reported an increase in the resting membrane potential (defined as the minimum recorded membrane potential during bursting) following knockdown of T-type Ca^{2+} channels in Drosophila R5 neurons. Since calcium currents are depolarizing, in the current work the involvement of Ca^{2+}-activated K^{+} currents was hypothesized. Simulations showed that such a channel could indeed negatively modulate the minimal membrane potential with reduced number of the T-type channels.

Transition between tonic spiking to bursting

Slow-freqeucny oscillations following Na^{+} channel blockade

Increase in resting membrane potential following T-type channel knockdown

R5 neurons: inhibition-induced bursters?

Conductance-based computational models

(Krumm 2021) implemented R5 and helicon cells based on Izhikevich model of burstin neurons (E. Izhikevich 2003).

(Krumm 2021) suggested the inhibitory synapses between R5 and helicon cells to explain this observation.

——————————— IMPORTANT!!!:

- Citations from Lauras thesis
  - In the Down state, Helicon is entrained to R5's compound rhythmicity via excitatory coupling. This leads to a relatively short offset (7 ms) between the two signals
  - For Helicon in the Down state, we find a much larger and negative offset of -77 ms (fig. 2.34a). We assume this is because Helicon now also receives inhibitory inputs from R5 neurons which prevent Helicon from firing and therefore lead to a small anti-phase correlation between the two signals.
- From Manuscript:
  - (Simulations). This is also in line with our experimental data, which show that the balance controls the degree of synchronization between excitatory and inhibitory

drive and determines whether the networks are in the shifted or synchronized configuration

- Remarks
  - In the Lauras thesis, in the second note it should be written ”Up State” instead of ”Down State”. However, this state corresponds to daytime rather than night. Thus this will not explain the experimental observations (shifted state at night)
  - In manuscript, 1) there is no inhibition from R5 to helicon at night. Thus, the temporal shift might be due to the synaptic time constant between helicon and R5, rather than interplay between excitation and inhibition between R5 and Helicon. Synaptic time constant was set to be 100 ms (similar to resulted time delay between helicon and R5). Thus, when additional input was provided to helicon, here helicon might drive R5 and R5 might burst due to intrinsic properties.

Facts:

- Ca1T-null mutants showed increased sleep (Jeong et al. 2015)
- Ca1T in drosophila are located at presynaptic terminals of R5

Discussion:

- Blocking NMDR - irregular interburst interval, controls - regular. Chaos??? (further support for square-wave) (Raccuglia et al. 2019; E. M. Izhikevich 2000)
- Although h current is associated with the repolarizing current (blcking sometimes reduces it), h current is not ncessary to observe this phase (examples of simulations).

  (Jeong et al. 2015) Flies lacking T-Type channels increase amount of sleep. If bursting in R5 requires sleep, than this is on the one hand counterintuitive result. Although, knocking down expression of T-Type channels in whole fly might have more complex effects on sleep, as other circuits that affect R5 acitivty also will lack the similar channel that might result in this observation.

(Blum et al. 2021): astrocytes and Toll receptors on R5 neurons. Toll might trigger gene expression (??? Need a bit more literature research)

BRP - Important for regulation of calcium channels (???)

RMP depolarized following SD (Connection to T-Type channels and increased RMP in knockdown. Effect of affector circuits following SD, as Ca gated K channels should have the opposite effect ???) (Liu et al. 2016)

- R5 neurons exhibit 1Hz tonic firing during day and 1Hz tonic firing during night
  - According to Liu et al 2016 bursting occurs only in sleep-deprived files (Liu et al 2016). However, Raccuglia et al 2019 reported bursting activity at the evening (ZT8-13). The difference might be because Liu et al reported above-mentioned results for ZT0. As R5 is modulated by both cyrcadian and homeostatic processes (circadian by clock neurons (Dopp et al. 2024)) - this might explain the difference.
  - Furthermore, I could not find the original paper stating that R5 neurons exhibit 1Hz tonic firing during day (Figures **??**, **??**)
- Raccuglia: frequency of R5 activation and locomotion. Actitation was done by optogenetics. If bursting is mediated by hyperpolarization activated current, then it can be that optogeneetically one directly activates fast system. Thus, you will need specific frequency of activation to induce similar effect (intrinsic bursting 1Hz).
- Other mechanisms are likely to be involved during normal, undisturbed sleep (Liu et al. 2016).
- Manuscript: "Because R5 activation can also entrain dFSB activity during the day (Extended Data Fig. 2a-c), we suspect that this interaction would effectively set helicon cells to the downstate (night setting), allowing for entrainment of helicon by R5."
  - This can also be due to DN1p clock neurons, not through dFSB
  - While helicon cells can be set to the downstate through DN1p-dFSB circuit, the SWA could be achieved through R5-helicon-dFSB circuit, where excitatory synapses from

helicon drive SWA in dFSB. It will be interesting to see the time lag correlation between dFSB-helicon and dFSB-R5 in the SD condition. Or even better - granger causality (as correlation does not tell us about causality). (Paper for method: Reassessing hierarchical correspondences between brain and deep networks through direct interface https://www.science.org/doi/10.1126/sciadv.abm2219)

Oscillations after TTX block - neuron might receive external input from other sources than synaptic current (e.g. gap junctions to other neurons or astrocytes - add citations)

Although for many bursting neurons H current is necessary (blocking of H current blabla, find literature) it is not mandatory for afterhyperpolarization (AHP) (image from Izhikevich book and models that have ahp but still have very nice bursts or ahp).

- In conntrast to three-compartment mmodel, single-compartment model could only estimate either I-V relationship, or LTS, but not both, with LTS observed in case of increased maximal conductance of T-Type channels. (Destexhe et al. 1998)

We assume that the R5 neurons are intrinsic bursters, i.e. they can exhibit bursting activity due to cell-autonomous conductances, even in case of constant input current. Thus, when concentrating on simulations of R5 neurons to study either transition between bursting and tonic spiking or effects of blocking specific ion channels, external input to R5 neuron is modelled as a constant. Furthermore, for simplicity, single compartment conductance based model was chosen. Such model 1) does not account for dendritic computations, and 2) assumes uniform distribution of ion channels. Nonlinear interactions in the multi-compartment model might better explain the experimental results without need of L-Type Ca^{2+} currents.

Different models

- Ion channels
- Same model, different parameter regimes
- If not mediated by t-type, they do not include T-Type channels
- Different goals: inhibition rebound vs induced bursting

- Slow variable for calcium oscillations
- Misreported parameters (HM neuron in paper and in one thesis)

Neurons differ by expression of ion channels, location of ion channels, concentrations. Thus, different models incorporate different ion channels and max conductances depending on neuron of interest

Single and three compartment neuron model - Destexhe (Destexhe et al. 1998).

- Turrigiano et al 1995 & Tang: `https://pmc.ncbi.nlm.nih.gov/articles/PMC6578228/pdf/jneuro_15_5_3640.pdf`
- Fazli et al 2020, Bertram et al 2000: phantom bursting + no sodium

EAG: different types (some has only activation, some does not, some are inactivated more by calcium, some not) - two papers on EAG

Do the simulations with noninstantaneous activation for Wang model - maybe the transition between spiking and resting will be easier

Maybe try to hand-tune one TTX oscillation and then use optimisation to fit others

It is hard to fit generally, as the ion channel dynamics overlap - e.g. extending time constant might affect other currents and fitting might be the best method

Did not incorporate calcium in day/night - calcium activates k dependent K channels + effectively modulates calcium channel strength -¿ Thus the effect might be even better

Thing to fit the neurons (blue thing, find what it was)

Slow oscillations in small parameter region - input current is important, as it only affects the V-nullcline. You change it, you move away - you move away from the slow manifold if the trajectory passes along it.

Fast activation of EAG to terminate burst after first spike - generally not required. It is just thought that the R5 fires tonically. However, it can be that it fires only few (e.g. 2) spikes per burst. The model tells, that it can be possible to reduce the number of spikes to 1. However data analysis should be done to see what is in R5 neurons.

switching from day to night will not depend on type of bursting

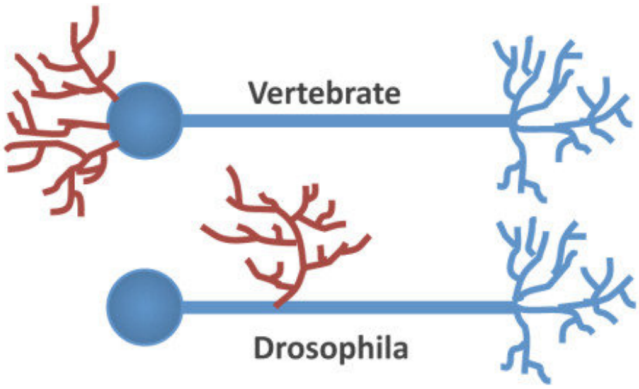

Figure 1: Comparison of neuron morphology in *Drosophila* and Vertebrates. In comparison to vertebrates, the neurons in *Drosophila* have unipolar morphology. Therefore synaptic potentials travelling from the dendrites (red) to spike initiation zone bypass cell body (blue circle). Figure adapted from (Spindler and Hartenstein 2011).

Different effects with changing one variable also is observed when two parameters are varied (my plot) :D Now you can imagine what will happen in higher dimensions :D

Slow calcium removal and bursting `https://pmc.ncbi.nlm.nih.gov/articles/PMC3650241/pdf/10827_2012_Article_430.pdf`

Slow kinetics via slow calcium removal: A Simple biophysically plausible model for long time constants in single neurons.

Although R5 neurons share similar functional characteristits with thalamic neurons, morphology of neurons in *Drosophila* is significantly different from those in vertebrates 1. Unlike vertebrates, *Drosophila* neurons have unipolar morphology. Because of this morphology, synaptic potentials travelling from dendrites to spike initiation zone bypass 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 (slecifically, Kenyon cell (KC)) are not solely postsynaptic, but also form presynaptic active zones (Christiansen et al. 2011).

- Estimated number of activation gates for Drosophila T-Type ion channels is 3.
- modeling the ion channel using Ohmic relationship between current and voltage did not procude good fits to observed current-voltage (I-V) relationship.

- The current-voltage relationship was reproduced when Goldman-Hodgkin-Katz (GHK) voltage flux equation was used instead of Ohmic current
- GHK equation models explicit relationship between current, voltage, temperature and intra-/extracellular ion concentrations.
- The fit of simulated I-V relatoinship to the observed data was improved when the steady-state activation function was shifted along V axis, and corresponding time constant was scaled and shifted along V axis (parameters for shifting and scaling were taken to be free parameters during optimization)