The role of dopaminergic neurons in the sleep maturation of Drosophila

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Abstract

Sleep is a deeply conserved and universal feature of many organisms, but the specific biological mechanisms remain poorly understood. We seek to understand why young animals sleep more than mature adult animals. Sleep is believed to play a specific role in brain development during the juvenile stage. Drosophila Melanogaster is used as a model organism to study genes involved in sleep. Studies show that the dopaminergic cells that synapse onto the sleep-promoting dorsal fan shaped body neurons of flies inhibit sleep output, thus promoting arousal. These dopamine neurons are less active in young flies, causing a more active sleep center. We aim to identify what controls this change in dopamine activity throughout fly maturation. Using preliminary results from single-cell RNA sequencing of changes in gene expression over the lifetime of a fly, we manipulated the expression of genes that are more highly expressed in mature flies and observed their sleep behavior through a behavioral screen using the GAL4-UAS system. “Hits” were defined as RNAi lines that increased sleep in mature adult flies. We found that the knockdown of mitochondrial genes regulating Complex I of the electron Transport Chain caused increased sleep in mature flies. Future work will focus on the biological significance of this complex as well as the general role of ATP production in sleep ontogeny.

Goal: Identify genes that drive maturation of sleep-relevant dopaminergic neurons

1. Introduction

Sleep & Ontogeny

The functions of sleep are mysterious, and the role of juvenile sleep is even more puzzling – for example, human infants must do critical things such as build social bonds, develop motor skills, and interact with their environments yet spend up to 18 hours a day sleeping. Although it is believed that early sleep is important for development, the neural mechanisms of sleep maturation remain unclear.

A powerful model to study this question is Drosophila Melanogaster. Similarly to humans, their sleep characteristics mature over time. This suggests that sleep is developmentally regulated, and sleep ontogeny is conserved across species.

Features of sleep that are observed in both flies and humans:

1) Behavioral quiescence
2) Postural changes
3) Reduced sensitivity to environmental stimuli
4) Homeostatic regulation
5) Rapidly reversible state
6) Changes to sleep across the lifespan

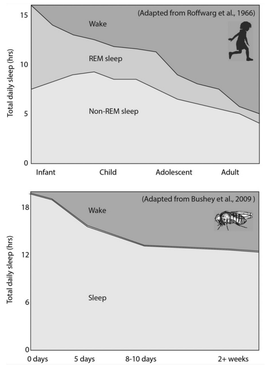
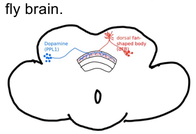


Figure 1. Maturation of sleep over time in humans and flies

The role of Dopamine in sleep regulation

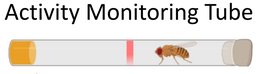
Dopamine is a conserved arousal cue from invertebrates to vertebrates. A group of dopaminergic neurons send inhibitory projections to a sleep promoting center of the central fly brain.



Measuring Sleep in Drosophila

Sleep = Bout >5 minutes of no beam breaks

Activity Monitoring Tube



High throughput analysis of sleep

2. Methods

Single cell RNA sequencing to determine target genes

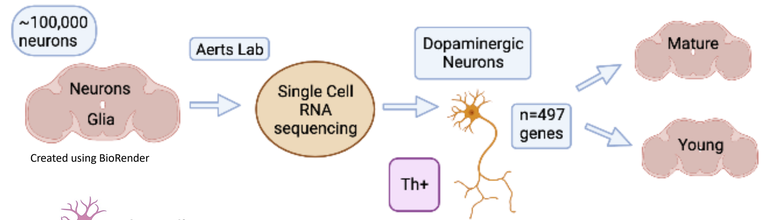
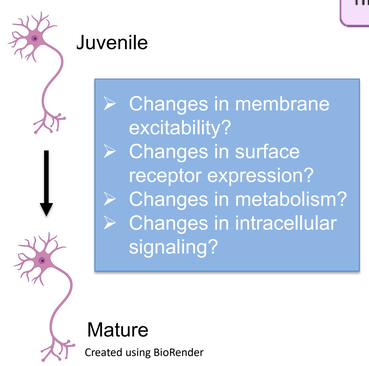


Figure 2. Differential gene expression analysis



- Changes in membrane excitability?
- Changes in surface receptor expression?
- Changes in metabolism?
- Changes in intracellular signaling?

Functional Class	Example Genes
Pathfinding	Ten-a, sdc, klingon
Membrane Trafficking	Rab5, Syt4, Vha26
Synapse Formation	Dpr1, Appl1, ben, Vap33
Neurotransmission	Gaba-t, Cadps, Ace, ChAT
Cytoskeletal regulation	Rac1, Futsch, Sif, Kank
Phosphorylation	SPRK, gish, Schip1, Mob2

Pilot RNAi screen in DANs

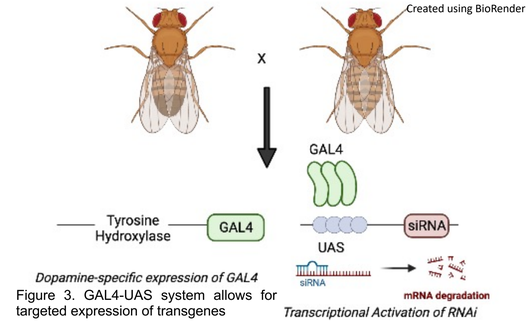


Figure 3. GAL4-UAS system allows for targeted expression of transgenes

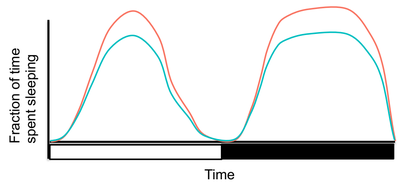


Figure 4. Sample sleep trace showing a control line (blue) and a line increasing sleep (red) over a 24 hour period of Light/Dark

3. Identification of RNAi conditions causing increased sleep in mature flies

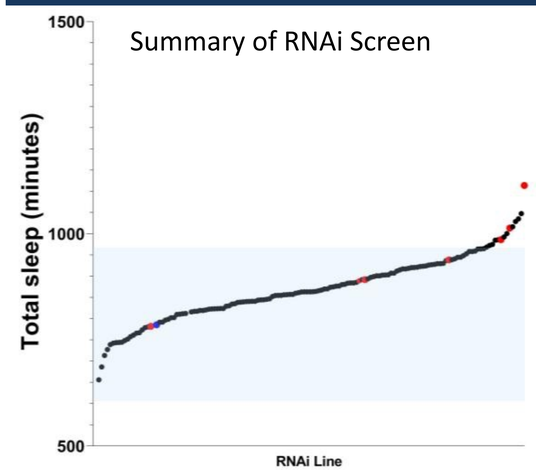


Figure 5. Plot of Total sleep for each RNAi line tested

- Measured sleep in 6-8 day old female flies in 12 hour light/dark cycle in 25 degrees Celsius for 149 target RNAi lines
- Total sleep was averaged across 3 consecutive days
- Data were analyzed using Rethomics
- Searched for conditions that gave extreme phenotypes rather than subtle changes

4. Depleting components of Complex I in Electron Transport Chain increases sleep in mature flies

Disruption of subunit of Complex I of ETC increases sleep

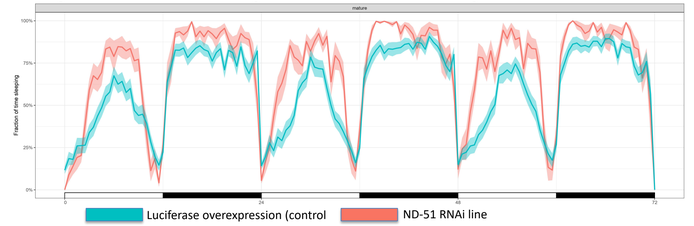


Figure 6. Sample sleep trace of ND-51, a protein coding gene in Complex I of ETC (red) and Luciferase-RNAi control (blue)

- 12 hour light period represented by the white bars, 12 hour dark period represented by black bars
- Knocking down ND-51 causes increased sleep

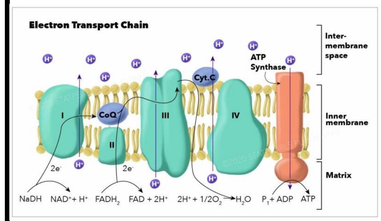


Figure 7. Electron Transport Chain

- A series of protein complexes that creates an electrochemical gradient to generate ATP through coupled redox reactions (oxidative phosphorylation)
- After finding the ND-51 hit, we were interested in the increased sleep phenotype being specific to that gene or related to Complex I of the ETC itself

Total Sleep for Complex I Targets

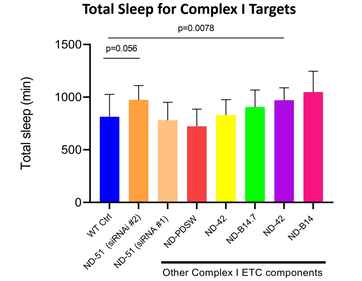


Figure 8. Total Sleep for Complex I targets
Complex I may be required for sleep maturation

Activity Indicies for Complex I Targets

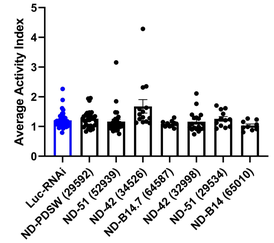


Figure 9. Activity Indicies for Complex I targets
Activity Index is defined as the number of beam breaks per waking time and acts as a measure of locomotor activity

Conclusions and Future Directions

- Identified RNAi conditions that increase sleep in mature flies
- Identified a potential role for Complex I of the ETC in functional maturation of dopaminergic neurons
- Future experiments will focus on the biological significance of Complex I as well as the general role of ATP production in sleep ontogeny
  - Are flies sleeping more due to a systemic effect on DANs or are a specific subset affected?
  - Is the phenotype caused by reduced complex I function throughout the lifetime or specifically in adult stages to regulate sleep?
  - Are there changes in oxidative phosphorylation in DANs as they mature?
  - We also knocked down components of ATP synthase and did not see a significant effect on sleep, suggesting that the failure of ATP production itself is not sufficient to explain the phenotype
    - Are changes in Reactive Oxygen species important to regulating DANs over time?

Acknowledgements

Davie K, Janssens J, Koldere D, De Waegeneer M, Pech U, Kreft Ł, Aibar S, Makhzami S, Christiaens V, Bravo González-Blas C, Poovathingal S, Hulselmans G, Spanier KI, Moerman T, Vanspauwen B, Geurs S, Voet T, Lammertyn J, Thienpont B, Liu S, Konstantinides N, Fiers M, Verstreken P, Aerts S. A Single-Cell Transcriptome Atlas of the Aging Drosophila Brain. Cell. 2018 Aug 9;174(4):982-998.e20. doi: 10.1016/j.cell.2018.05.057. Epub 2018 Jun 18. PMID: 29909982; PMCID: PMC6086935.

Kayser MS, Biron D. Sleep and Development in Genetically Tractable Model Organisms. Genetics. 2016 May;203(1):21-33. doi: 10.1534/genetics.116.189589. PMID: 27183564; PMCID: PMC4858775.