A candidate-based screen to identify genes involved in sleep maturation

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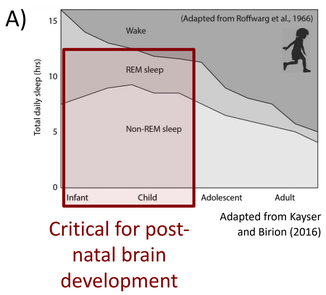
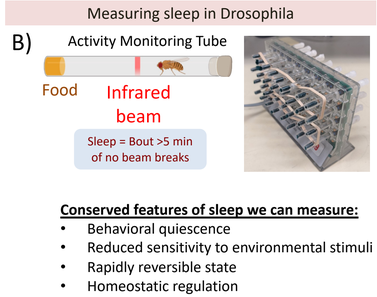
Abstract

Sleep is a universal behavior among animals, despite leaving them defenseless and unable to carry out essential activities – suggesting an essential function. In early life, sleep is thought to promote brain and behavior maturation. Consistent with a privileged function, sleep in early postnatal life is qualitatively distinct from adult sleep, characterized by increased sleep duration, depth and architecture. The molecular control mechanisms underlying juvenile sleep and sleep maturation are poorly defined. The vinegar fly *Drosophila melanogaster* is a powerful organism to study juvenile sleep due to its wealth of tools to allow gene manipulation. Like humans, juvenile adult flies (0-1 days after eclosion) sleep longer, and more deeply, than mature adults (~1 week old). Moreover, sleep in *Drosophila* is regulated by conserved neurotransmitter systems, including dopamine (DA), an arousal-promoting cue. Previous work demonstrated that sleep maturation is controlled by changes to DA tone, resulting in increased arousals during sleep during the juvenile to mature transition. The molecular basis of this change remains entirely uncharacterized. To identify genes acting in adult dopaminergic neurons (DANs) to drive sleep maturation, we hypothesized that changes in gene transcription in DANs cause changes in DAN activity to drive sleep maturation. To test this hypothesis, we identified differentially expressed genes between juvenile (0-1 day old) and mature (6 and 9 day old) DANs from publicly available scRNA seq data of the adult fly brain. Candidate genes were systematically knocked-down in DANs using short hairpin RNAs to screen for conditions that caused sleepy mature flies, suggesting a potential defect in sleep maturation. To enrich for genes acting preferentially in juvenile DANs, we followed up positive hits using a temporally-restricted knockdown approach. Using these techniques, we identified a novel role in sleep maturation for Ringmaker, the sole *Drosophila* homolog of the mammalian Tubulin Polymerization Promoting Protein. These experiments suggest an underappreciated role for microtubule cytoskeleton in the maturation of sleep circuits.

Introduction

Sleep characteristics mature over time





Conserved features of sleep we can measure:

- Behavioral quiescence
- Reduced sensitivity to environmental stimuli
- Rapidly reversible state
- Homeostatic regulation
- Changes to sleep across the lifespan

Figure 1. (A) Sleep ontogeny changes across a human lifespan. (B) Drosophila activity monitoring system for high throughput analysis of fly sleep

Dopamine regulates sleep maturation

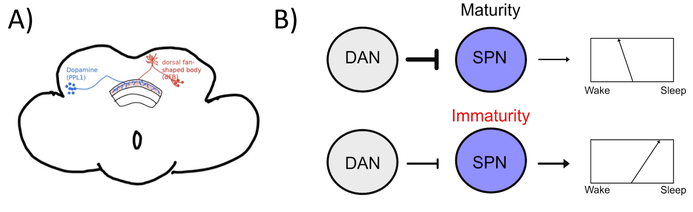


Figure 2. (A) Dopamine is a conserved arousal promoting cue. In the central fly brain, DANs (blue) make inhibitory connections to a sleep homeostat (red). (B) As a fly matures, dopaminergic tone on sleep promoting neurons increases, leading to higher inhibition of SPNs and thus increased wakefulness (1).

Methods

A screen to identify candidate maturation-promoting genes

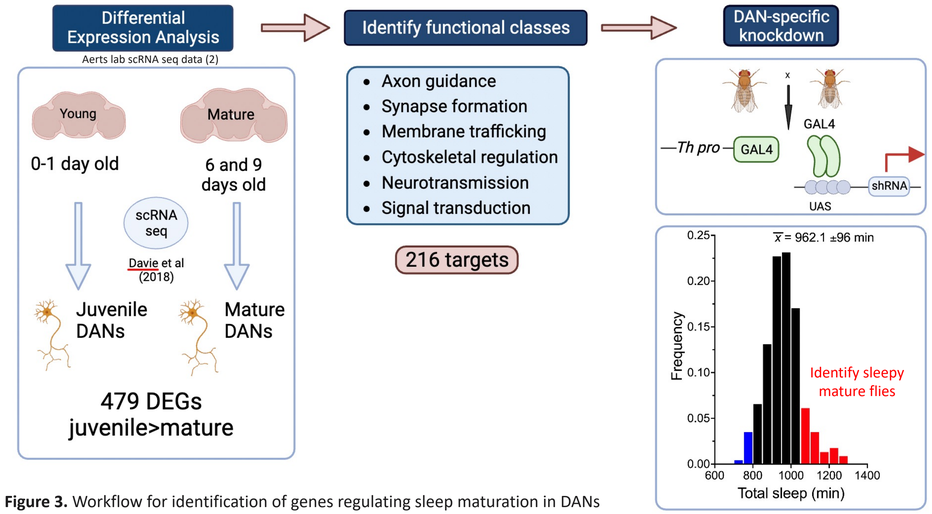


Figure 3. Workflow for identification of genes regulating sleep maturation in DANs

Temporally restricted knockdown to identify genes acting in adulthood

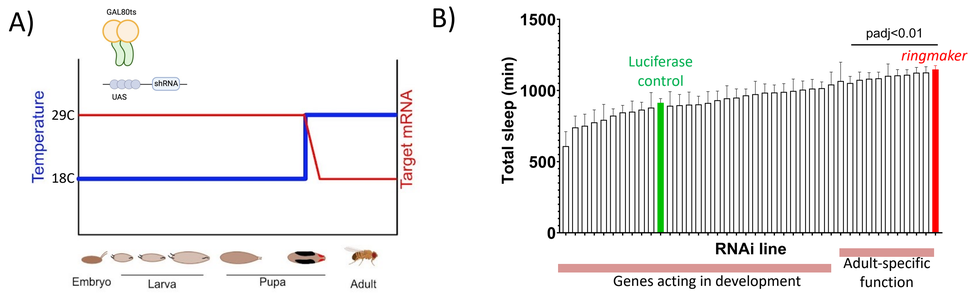


Figure 4. (A) To limit candidate gene knock-down to adult stages, a temperature-sensitive GAL80 was expressed ubiquitously to inhibit GAL4-dependent shRNA expression during development at a permissive temperature (18C). Flies were shifted to the non-permissive temperature (29C) as late pupae. (B) Total sleep is plotted for conditional knock-down of hits from primary screen. 9 genes were identified to have an adult-specific function in DANs. Data were analyzed using a Kruskal-Wallis test.

ringmaker is required in adult DANs to limit sleep

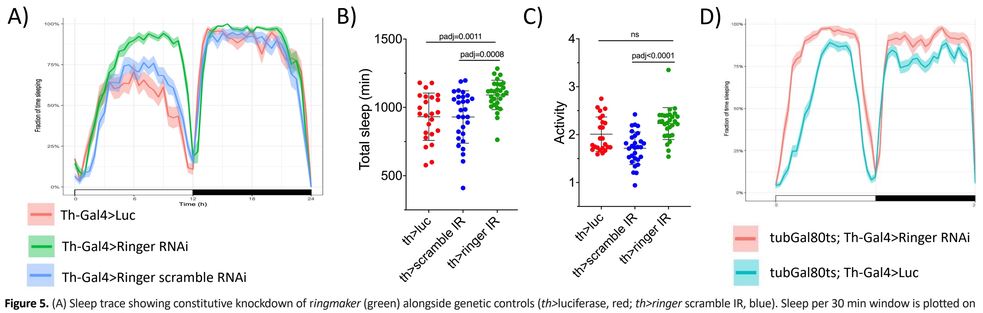


Figure 5. (A) Sleep trace showing constitutive knockdown of *ringmaker* (green) alongside genetic controls (*th*>luciferase, red; *th*>*ringer* scramble IR, blue). Sleep per 30 min window is plotted on the y axis for a 24h light-dark period. Depletion of *ringer* in DANs causes a significant increase in sleep, most notably day sleep. (B) Total sleep plotted for each genotype from the sleep trace in (A). Data were analyzed using Brown-Forsythe test and Welch ANOVA. (C) Activity index (beam breaks/ waking time) is plotted for each genotype showing increased sleep in *th>ringer* IR is not caused by changes to locomotor activity. Data were analyzed using a Kruskal-Wallis test. (D) Sleep trace showing temporally restricted adult knockdown of *ringmaker* (red trace) with a genetic control (*th*>luciferase, blue trace). Restricting *ringer* depletion to adult DANs is sufficient to recapitulate hypoarousal phenotype seen by constitutive knockdown (A-C).

ringmaker is required for sleep maturation

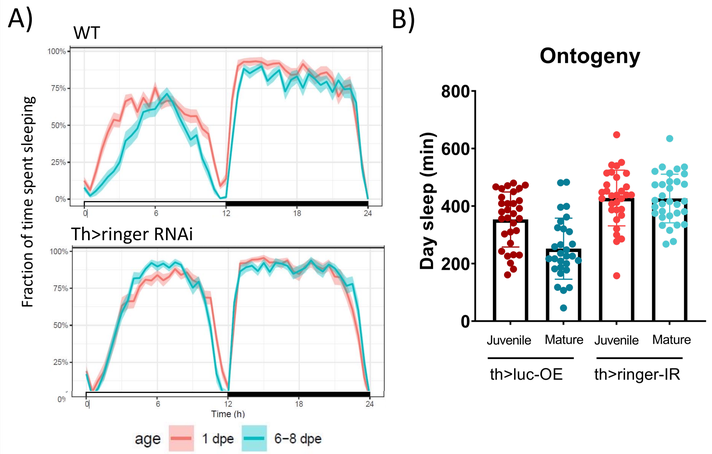


Figure 6. A) Sleep trace showing juvenile (1 day post-eclosion) and mature (6-8 dpe) wild-type (WT, top trace) and Th>ringer RNAi (bottom trace) flies. B) Plot of day sleep for juvenile and mature control and th>ringer-IR flies. Juveniles show elevated sleep compared to mature controls, however this phenotype is abolished when ringer is depleted.

Is microtubule regulation important for sleep maturation?

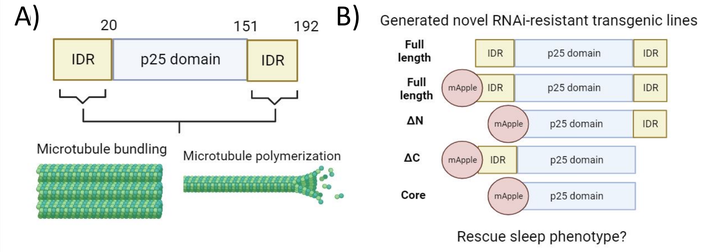


Figure 7. A) Ringmaker is the Drosophila homolog of Tubulin Polymerization Promoting Protein/p25. At its N- and C-termini are intrinsically disordered regions (IDRs) that promote microtubule polymerization and bundling (3). B) To assess the contribution of these microtubule regulating activities, I generated UAS-dependent transgenes encoding mApple-tagged RNAi resistant deletion constructs

Conclusions and Future Directions

Conclusions

- Conducted a biased screen to identify genes acting in DAN maturation based on an increased sleep phenotype in mature flies
- Identified 9 genes acting specifically in adult flies using a temporally restricted knockdown approach
- Identified a role and established the requirement for *ringmaker*, a gene regulating microtubule stabilization, in sleep maturation

Future directions

I. Conduct further behavioral analysis of increased sleep phenotype failure in sleep maturation
   I. Differentiate between loss of ontogenetic change in sleep bout number, duration, or arousal threshold

II. Determine the microtubule defects that underlie Ringmaker loss of function phenotype
   I. Possibly defects in microtubule dynamics or microtubule-based transport
   II. Will pursue these phenotypes using live imaging

References

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