# Unraveling the Secrets of Sleep in *Drosophila*: Role of Methyltransferase-like 3 on Sleep Regulation

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## Introduction

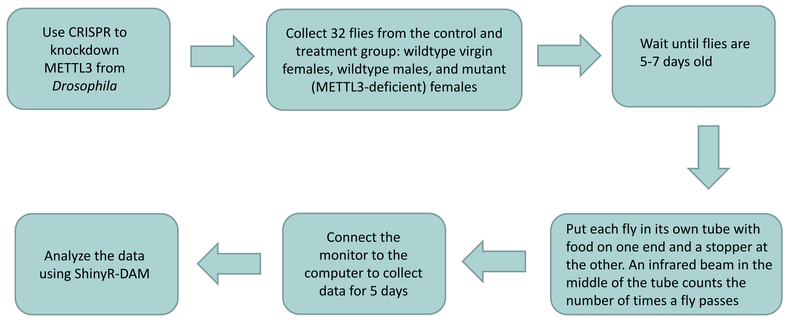
Sleep is a critical phenomenon in *Drosophila* to maintain biological functions including, circadian rhythm, reproduction, stress resistance, development, learning and memory, and homeostasis[1]. Disruptions in sleep can become detrimental and affect many biological functions. Methyltransferase like 3 (METTL3) is a catalytic subunit that deposits the m6A modification at its specific target sites in mRNA[4]. $N^6$-methyladenosine ($m^6A$) is one of the most abundant modifications of mRNA that performs a variety of significant biological functions in brain development and maturation critical for the circadian rhythm[2]. This study investigates the role of METTL3 in regulating sleep in female *Drosophila*. Previous research has shown that METTL3 knockdown can elongate cell cycle progression, compromise memory and hinder neuronal state[3].

## Objective

- Explore the effects of m6A depletion on sleep by METTL3 knockdown.
- Understand the sex specific differences in sleep patterns

## Methods and Materials

- All flies were cultured and reared at 25°C on a 12:12 h light:dark cycle.
- Sleep was measured through locomotion: Inactivity for 5 minutes or more is a proxy for sleep in *Drosophila*



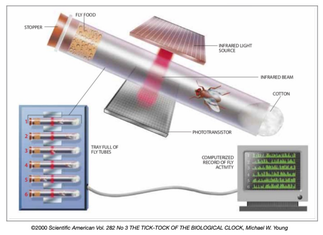




**Figure 1. A labelled diagram of each tube in the Drosophila activity monitor**

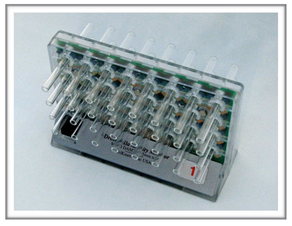


**Figure 2. An image of one of the *Drosophila* activity monitors.**

## Results

Mean Locomotion Activity

- There is no significant difference between WT males and females
- There is no significant difference between WT females and mutant females

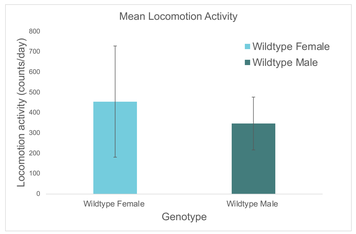



**Figure 3a. Mean locomotion activity of wildtype flies**

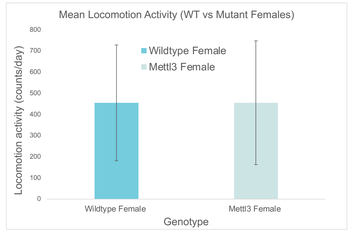



**Figure 3b. Mean locomotion activity of wildtype and mutant females**

Mean Sleep

- Females sleep less than males in wildtype conditions
- Mutant females sleep more than wildtype females

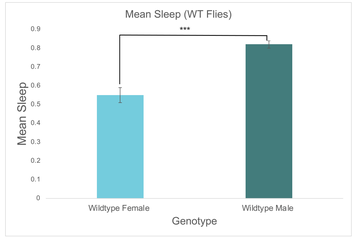



**Figure 4a. Mean sleep of wildtype flies**

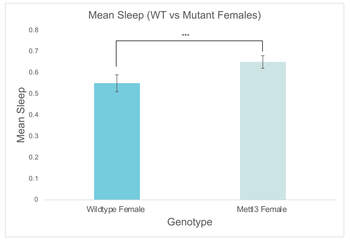



**Figure 4b. Mean sleep of wildtype and mutant females**

Mean sleep bout length

- In normal conditions, males have a greater bout length than females
- Mutant females have greater bout length compared to wildtype females

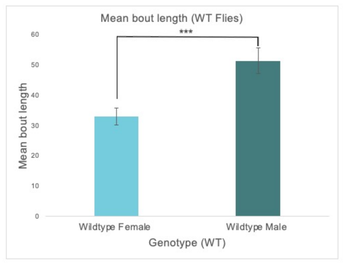



**Figure 5a. Mean bout length of wildtype flies**

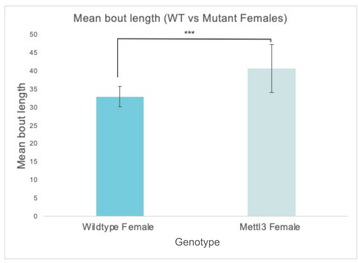



**Figure 5b. Mean bout length of wildtype and mutant females**

Mean sleep bout number

- In normal conditions, females have more sleep bouts than males
- Mutant females have more sleep bouts than wildtype females

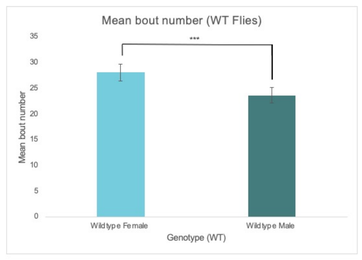



**Figure 6a. Mean bout number of wildtype flies**

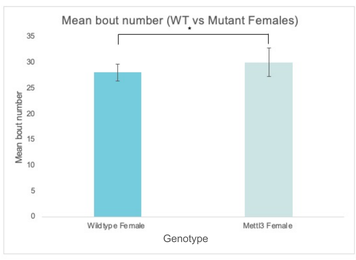



**Figure 6b. Mean bout number of wildtype and mutant females**

## Discussion

- There are sex specific differences associated with sleep patterns of *Drosophila*. Wildtype females sleep less than wildtype males (Figure 4a). Figure 6b and 6c show that wildtype females have a greater number of sleep bouts but sleep for a shorter amount of time in each bout. Whereas wildtype males have the opposite effect with a smaller number of sleep bouts but a greater length of each bout. These results showcase the differences between sexes in the control condition, hence, the appropriate comparisons should be made between the mutant and wildtype conditions (looking at either female or male).
- In Figure 5a and 5b, there are no significant differences between wildtype and mutant conditions which indicates that locomotion differences could not influence the differences in sleep.
- The knockdown of METTL3 had significant effects on the sleep patterns of female *Drosophila.* Figure 4b shows that METTL3-deficient females had an increased amount of sleep compared to the wildtype.
- METTL3-deficient females have a greater number of sleep bouts and longer lengths of each bout compared to the wildtype condition (Figure 5b and 6b).
- These differences between the wildtype and mutant conditions showcase the significant role of METTL3 on regulating sleep in *Drosophila*

## Conclusion

- METTL3 plays a critical role in the sleep regulation of *Drosophila*
- Controlled expression of m6A writer, METTL3, is required for proper circadian rhythm and sleep behaviors of *Drosophila*
- Knockdown of METTL3 is associated with changes in mean sleep, sleep bout length and number
- For future considerations, it would be beneficial to observe the effects of METTL3 in learning and memory, as sleep is associated with neurological processes[3]

## References

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2. Jiang, X., Liu, B., Nie, Z., Duan, L., Xiong, Q., Jin, Z., Yang, C., & Chen, Y. (2021). The role of M6A modification in the biological functions and diseases. *Signal Transduction and Targeted Therapy*, *6*(1). https://doi.org/10.1038/s41392-020-00450-x
3. Liu, P., Li, F., Lin, J., Fukumoto, T., Nacarelli, T., Hao, X., Kossenkov, A. V., Simon, M. C., & Zhang, R. (2021). M6A-independent genome-wide Mettl3 and METTL14 redistribution drives the senescence-associated secretory phenotype. *Nature Cell Biology*, *23*(4), 355–365. https://doi.org/10.1038/s41556-021-00656-3
4. Wang, Y., Li, J., Zhao, B., Huang, G., Li, X., Xie, Z., Zhou, Z., & Li, L. (2021). The emerging role of M6A modification in regulating the immune system and autoimmune diseases. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.755691

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