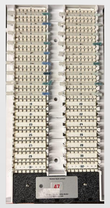
Is Cryptochrome Essential for Increased Sleep in Response to Blue Light in *Drosophila melanogaster?*

Gauri Paul, Ivan Soto, Lesly N. Soriano, Cynthia T. Hsu
Department of Biology, College of Science and Mathematics, California State University, Fresno

Introduction

- Cryptochrome (CRY) is a blue-light sensitive photoreceptor that has been associated with entrainment of the circadian rhythm (Abhilash et al 2022, Emery et al 2000).
- Previous work in our lab has shown that blue light promotes sleep in *Drosophila melanogaster*.
- We used two genetic approaches to determine the role of cryptochrome in the increased sleep in response to blue light
  - cry^{02} (*cryptochrome* loss of function) mutants
  - Expression of the inward rectifying potassium channel (KIR2.1) in CRY+ cells to silence cellular tarnsmission. Expression was suppressed until adulthood using the auxin-inducible gene expression (AGES) system.

Methodology



- For all experiments, flies were placed in multibeam *Drosophila* Activity Monitors and kept inside an incubator to maintain constant temperature and controlled lighting conditions.
- To temporally control transmission in CRY+ inward rectifying potassium channels, flies were treated with different concentrations of auxin (5 mM, 2 mM, and 1 mM) to induce GAL4 transcriptional activation.
- In experiments using 5 mM auxin, flies were exposed to a lighting paradigm (used previously by the lab) of three days of 12 hours of blue light followed by 12 hours of darkness (12:12 Blue:Dark), then three days of constant darkness.
- For all other experiments, flies were exposed to three days of constant darkness, followed by one day of 12:12 Blue:Dark.
- **Sleep** was defined using the historical definition of 5 min of inactivity (Hendricks 2000, Shaw
- **Death** was defined as any instance where a fly was inactive for at least 12 hours at the end of the six day activity recording period.
- **"Severe locomotor impairment"** was initially defined as any instance where less than 2 minutes of activity were observed per 30 min bin (5 mM condition). However, we later observed some instances where flies would become inactive for 24 hours, then move for 25 minutes in a 30 min bin, then become inactive again, we redefined "severe locomotor impairment" as instances when flies became inactive for at least 25 minutes in a 30 minute bin for an extended (longer than 24 hour) period of time (1 mM and 2mM conditions).
- Significant differences in death and severe locomotor impairment were computed using the logrank test with pairwise comparisons corrected by the Bonferroni method.

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Results

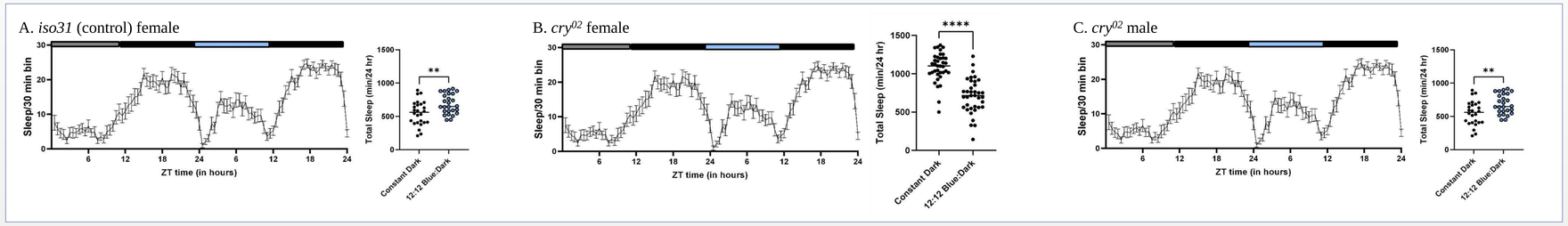

Figure 1. Unlike control flies, which increase their sleep when transitioning from constant dark to 12:12 Blue:Dark (A, female only shown, n=26), female cry^{02} (B, n=37) and male cry^{02} (C, n=32) mutants show a decrease in sleep when transitioning to blue light. For all panels, left side shows means and SEMs of sleep in 30 minute bins distributed over the third day of constant dark and the first day of 12:12 Blue:Dark. The right side shows the total sleep for individual flies, with the median represented by the horizontal line. ** represents p<0.01, **** represents p<0.0001 using the Mann-Whitney test.

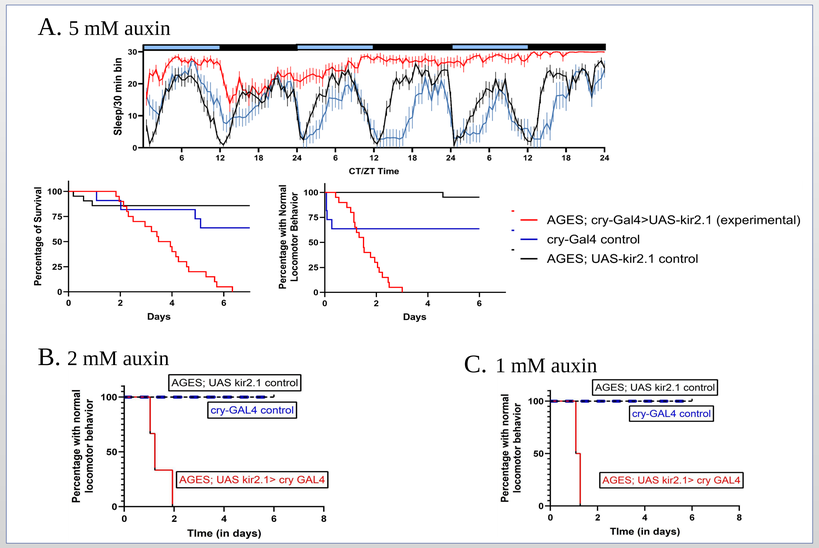

Figure 2. Experimental female flies showed severe locomotor impairment in response o KIR2.1 expression. Flies fed This is true regardless of whether flies were fed 5 mM **(A)**, 2 mM (**B**), or 1mM (**C**) of auxin. (**A)** On 5 mM, all female flies showed severe locomotor impairment (top panel, bottom left) followed by death (bottom right). Top panel shows means and SEMs per genotype of time spent sleeping in 30 minute bins. p<0.001 difference between all three genotypes. p<0.001 for the pairwise difference between *AGES; UAS-kir2.1* control (n=21) and the experimental (n=20). p<0.01 for the pairwise difference between *cry-Gal4* control (n=11) and the experimental. **(B)** On 2mM auxin, significant differences (p<0.05) in time to severe locomotor impairment were observed for all three genotypes, although there were no significant differences in pairwise comparisons, likely due to a low number of flies (n=2-3 per genotype). **(C)** On 1 mM auxin, significant differences in time to severe locomotor impairment were observed for comparisons between all three genotypes (p<0.01), between *AGES; UAS-kir2.1* control (n=3) and the experimental (n=3) (p<0.05), and between the *cry-Gal4* control and the experimental (n=3).

Conclusion

- Functional cryptochrome is necessary for blue light to drive sleep increases
- Expression of KIR2.1 in CRY+ cells is lethal regardless of concentration of auxin used
- Effects of KIR2.1 in CRY+ cells are sexually dimorphic:
  - Males are less likely to survive KIR2.1
  - Females, but not males, undergo a long period of locomotor impairment prior to dying following KIR2.1 expression.

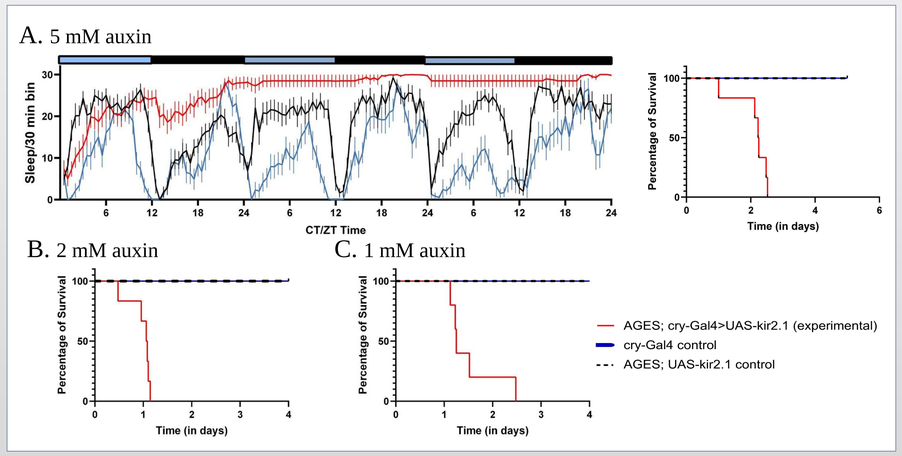

Figure 3. Male flies did not show any locomotor impairment but died soon after the expression of KIR2.1. (A) 5 mM auxin. Top panel shows means and SEMs for 30 min bins on the third day of constant darkness and fourth day of 12:12 Blue:Dark. Bottom panel shows fraction surviving. p<0.0001 for all genotypes and all comparisons. **(B)** Fraction surviving. 2 mM auxin. p<0.0001 for comparisons between all genotypes. p<0.01 difference when pairwise comparison is made between experimental flies (n=6) and either the *AGES; UAS-kir2.1* control (n=5) or the *cry-Gal4* control flies (n=5) and experimental flies (N=6). **(C)** For 1 mM, no statistically significant difference in time until death was observed (p = 0.0731, n=5 for all genotypes).

Future Directions

Our future goal is to determine if cryptochrome is developmentally necessary or if acute removal or silencing in adults is sufficient to prevent the sleep increase. We will use the following strategies:

- Use *nsyb-GeneSwitch* to selectively alter cryptochrome expression in the neurons of adult flies by driving expression of either
  - *cryptochrome* RNA interference (RNAi) to reduce mRNA expression, or
  - CRISPR-cas9 to selectively delete the *cry* gene in neurons.
- Since *cryptochrome* is expressed in the malpighian tubules, use *tsh-GAL80* to suppress KIR2.1 expression outside the brain and prevent lethality due to a possible renal failure.

We will consider the following factors in further experiments with *cry* mutants:

- does age of adult flies matter?
- does social isolation or overcrowding play a role in behavior patterns?
- how important is the intensity of blue light?

References

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