Circadian rhythmicity and neurodevelopment of *disco* and *grim* mutations in *Drosophila melanogaster*

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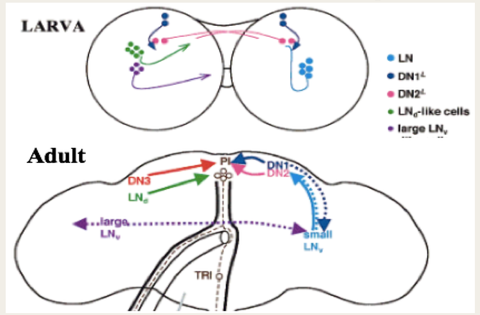
Abstract

The death gene *grim* and its pathway for apoptosis has been studied extensively in *Drosophila Melanogaster*. The effects of *grim* mutations on circadian neurodevelopment and locomotor assays have yet to be investigated. Mutations in the gene *disconnected* (*disco*) has been shown to disrupt the normal development of the circadian circuitry, specifically the small ventro-lateral neurons (*s*-LN_v's). Which has shown to severely decrease rhythmicity during free-running periods. Alternatively, we have observed an increase in rhythmicity during free-running periods in *grim* mutations. Our goal is to investigate the neurodevelopment of the circadian circuitry and their associated locomotor activities in these *Drosophila* mutations.

Circadian Circuitry



Kaneko M., and Hall J.C. (2000) Neuroanatomy of cells expressing clock genes in Drosophila: transgenic manipulation of the period and timeless genes to mark the perikarya of circadian pacemaker neurons and their projections. J. Comp. Neurol

Methods

To investigate the effects of these mutations on neurodevelopment, fluorescent spectroscopy using the UAS-gal4 system or antibody staining if a gal4 driver was not available was employed. Locomotor activities were recorded through a 3-day 12 hour LD entrainment period followed by a 7-day DD free-running period. All observations were compared with a control wild-type *w1118*.

Results

Figure 1

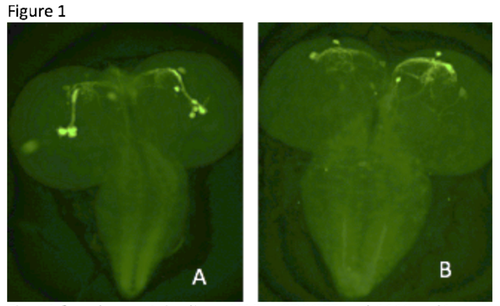


Fig. 1A females contain *disco, UAS-mCD8GFP* and *1118-gal4*
Fig. 1B males contain *disco', UAS-mCD8GFP* and *1118-gal4*

Figure 2

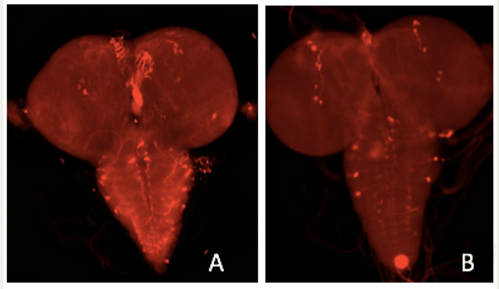


Fig. 2A *UAS-miD1T3,D2TC* (male) (X) *tub-gal4* (females)
Fig. 2B WT *w1118*

Table 1

Genotype	WT w1118	UAS-miD1t3,D2tc (female) ⊗ WT	UAS-miD1T3,D2tc (female) ⊗ tub-gal4	UAS-miD1s3,D2sa (male) ⊗ tub-gal4	UAS-miD1s3,D2sc (male) ⊗ tub-gal4
Mean +/- SD	4 +/- 0	4 +/- 0	2.9 +/- 0.94	3 +/- 0.66	3 +/- 0.63
n	12	12	11	10	6

Figure 3

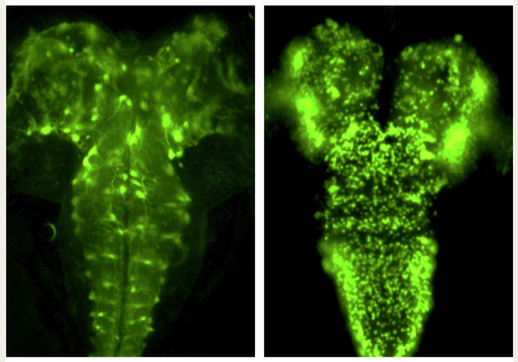


Fig 3A UAS-mCD8GFP X grim-gal4
Fig 3B UAS-greenstinger X grim-gal4

Figure 4

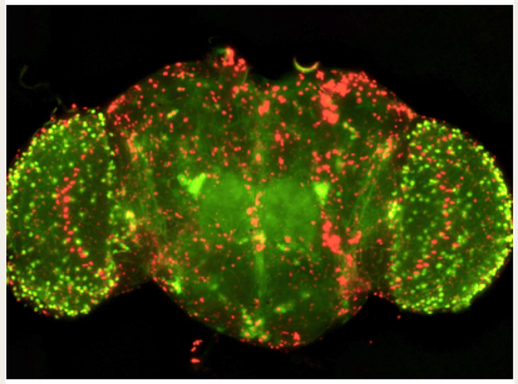
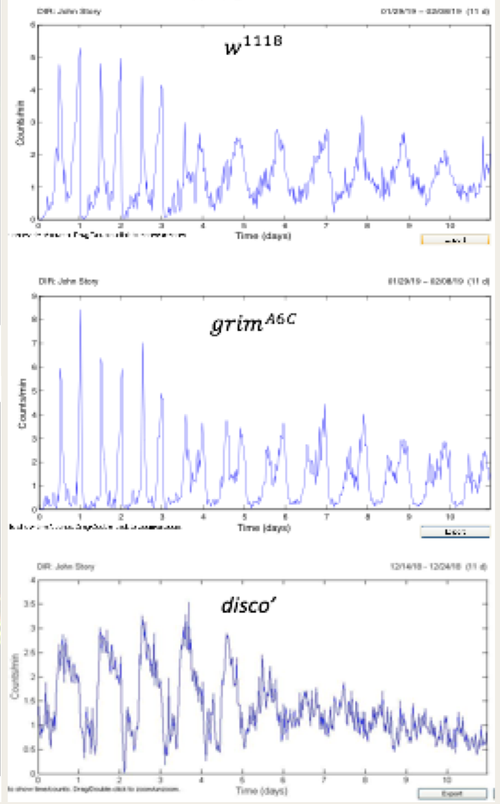


Fig. 4 UAS-mCD8GFP; UAS-redstinger X UAS-mCD8GFP; tim-gal4

Figure 5
Time series of wild-type, grim and disco mutants



Conclusion

- *disco* mutations cause an absence of *s*-LN_v formation and a loss of circadian rhythmicity during DD cycles.
- When the disconnected protein is suppressed by disco mi-RNA, intermediate levels of *s*-LN_v's develop.
- The expression of *disco* mi-RNA, however, is not sufficient to induce circadian arrhythmicity.
- While *grim* mutations have shown profound effects, strengthening circadian rhythmicity, we have not yet observed differences in neurodevelopment via fluorescent spectroscopy.

Future Research

- Future works will include repeating locomotor assays on *grim* mutants.
- Explore the expression of several circadian related proteins (*clock, pdf, cycle,* etc.) in *grim* mutants at different stages of development to observe how neurons in the circadian circuitry are effected.

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