Central Clock Control of Drosophila Behavioral Rhythms

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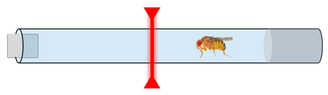
Introduction

The circadian system produces ~24-hour cycles in diverse biological processes. We are interested in understanding how the circadian system regulates different outputs, including locomotor activity and feeding behavior. The *Drosophila* brain contains ~150 central clock cells that keep time through a cell-autonomous molecular clock. These cells are subdivided into anatomically and functionally discrete neuronal clusters. We have used genetic tools to electrically silence different clock cell populations in flies and monitored the effect on feeding behavior and locomotor activity. We find that free-running feeding:fasting rhythms and rest:activity rhythms both require neuronal communication within multiple individual clock cell populations. Furthermore, the severity of the effect of neuronal silencing varies according to the cell population targeted. Our results show that central clock cells regulate feeding and locomotor activity rhythms in parallel, suggesting that circadian control of these two distinct behavioral outputs diverges in downstream circadian output cells rather than in cells of the core clock network.

Methods

Locomotor Activity Assay

The *Drosophila* Activity Monitor (DAM) system was used to record rhythms of activity. Male flies 7-10 days old were individually placed in glass tubes in the DAM, and activity was measured via infrared beam breaks for 10 days. Flies were entrained in 12hr:12hr light-dark (LD) conditions prior to experiments. Rhythm power is determined through the ClockLab analysis software (Actimetrics).



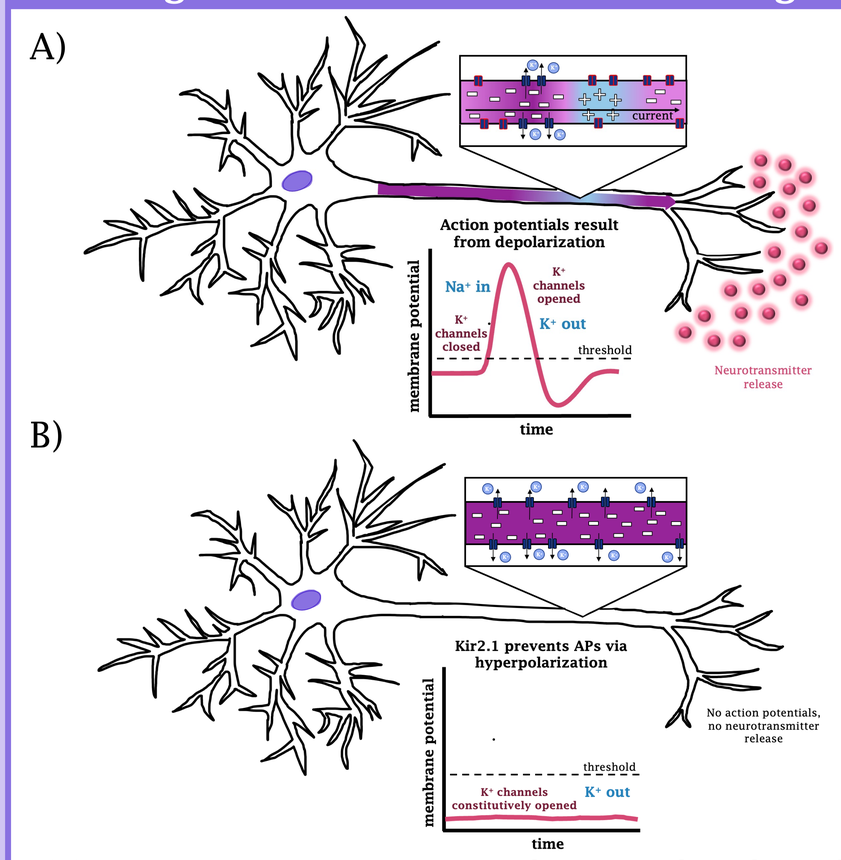
Feeding Behavior Assay

Feeding behavior was monitored using the Fly Liquid-Food Interaction Counter (FLIC) using 7-10 day old male flies for 7 days in complete darkness (DD). FLIC detects and records fly interactions with food (10% sucrose solution). Flies used in this assay were first entrained in 12hr:12hr light-dark (LD) conditions. Rhythm power is determined through the ClockLab analysis software (Actimetrics).

Acknowledgements

This work was supported by the National Science Foundation Division Of Integrative Organismal Systems, CAREER Award 1942167 to D.J.C

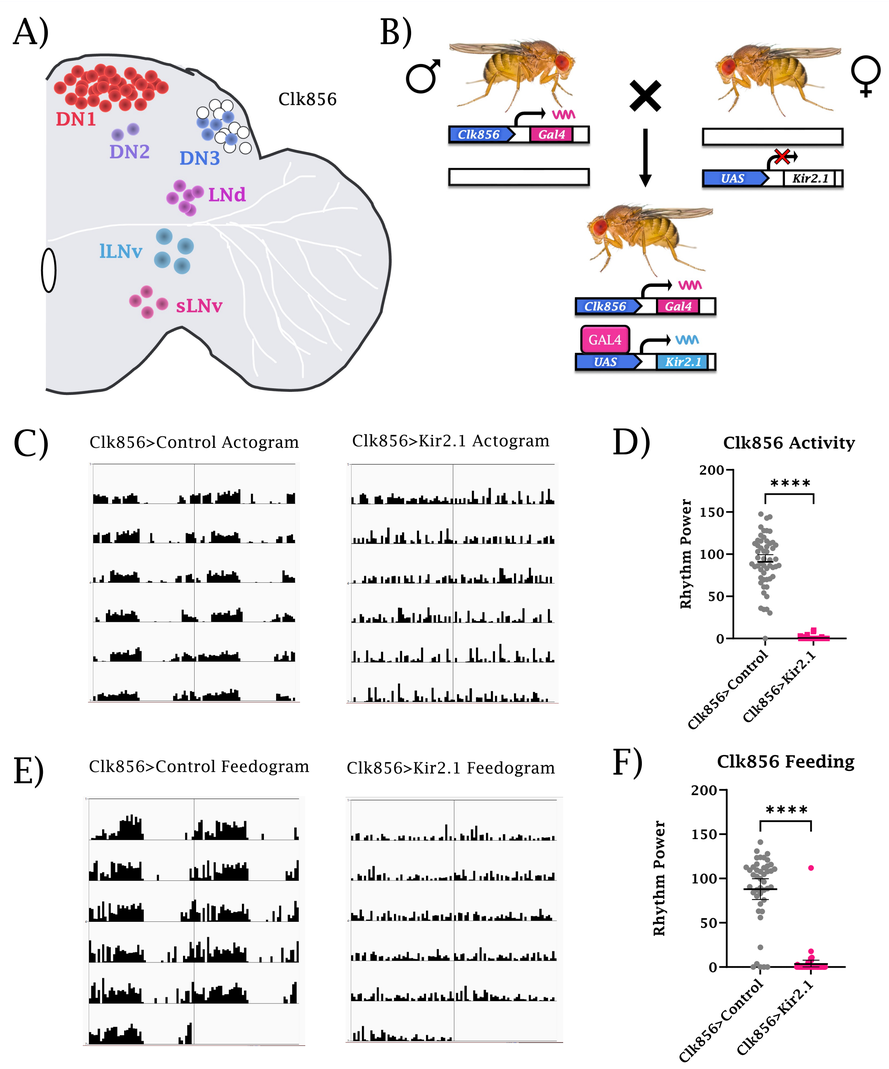
Figure 1: Kir2.1 Electrical Silencing



(A) Functioning neurons require voltage-gated K^+ channels that remain closed while cells depolarize

(B) Kir2.1 is a permanently opened K^+ channel that enables K^+ ions to flow out of the cell according to their electro-chemical gradient, suppressing neuronal excitability

Figure 2: Cell-Specific Electrical Silencing



A) Organization of the subsets of neurons in the central clock network, highlighted cells contain the cell-specific promoter *Clk856*

B) Schematic for genetic crosses that produce offspring that express Kir2.1 selectively in clock cells

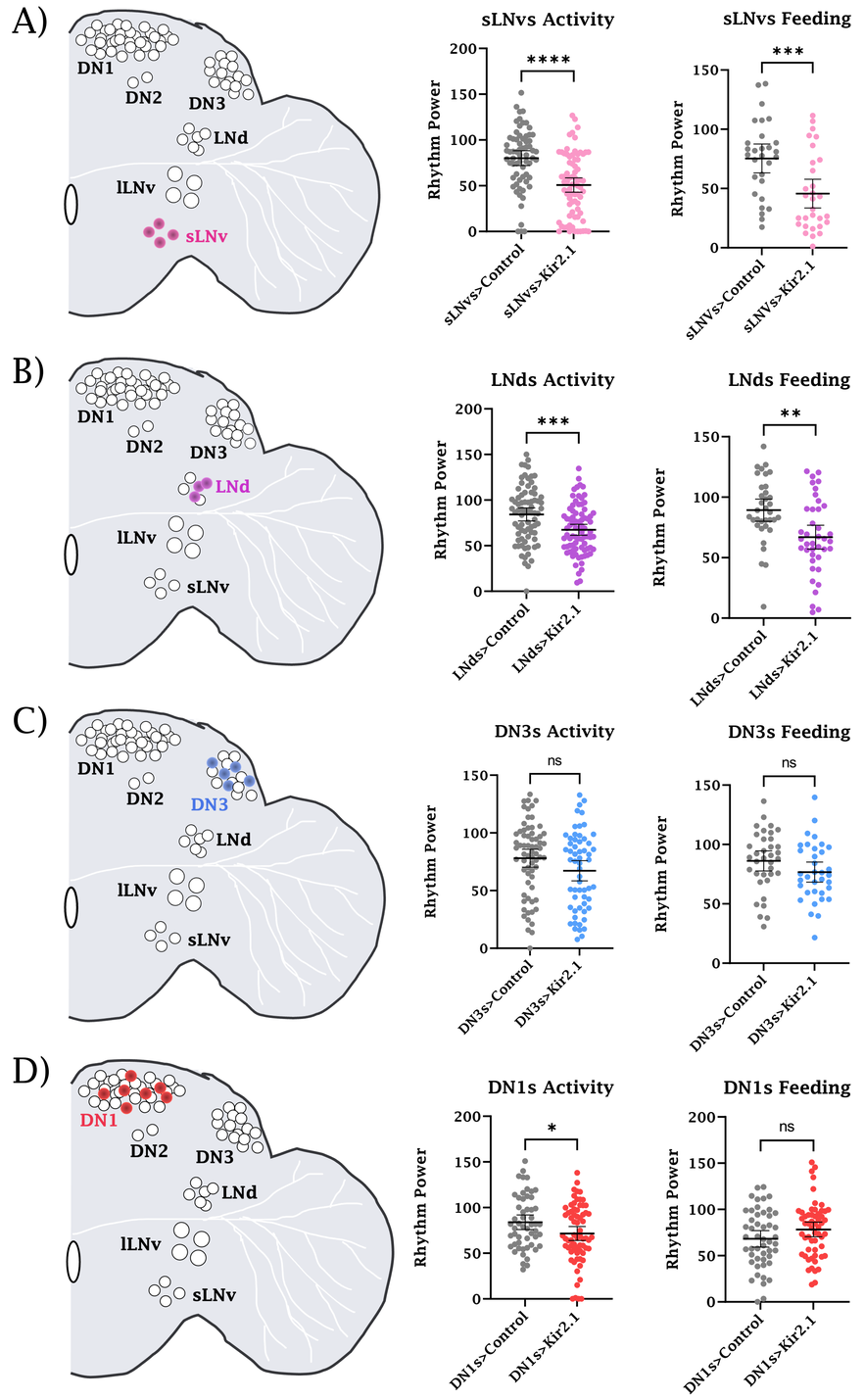
C) Representative images of flies' activity patterns with and without Kir2.1 expressed in clock cells

D) Average power of activity rhythms

E) Representative images of flies' feeding patterns with and without Kir2.1 expressed in clock cells

F) Average power of feeding rhythms

Figure 3: Subsets of Clock Cells Differentially Affect Rhythmicity



(A-D) Graphs show rhythm strength of control flies (gray) compared to flies in which Kir2.1 has been expressed in the cell populations indicated

Conclusions

- ❑ Feeding rhythms and activity rhythms both require neuronal communication within the clock network
- ❑ Neuronal silencing of sLNvs and LNds has a more detrimental effect on rhythmicity than DN3s and DN1s for both feeding and locomotor activity
- ❑ Circadian control of feeding and locomotor activity diverges downstream of the clock network

Future Directions

- ❑ Investigate neuronal populations downstream of the clock network and look for differential effects on feeding and activity rhythms
- ❑ Test behavioral rhythmicity of flies with mutated versions of the DH44 peptide and DH44 receptors
- ❑ Define the overall circuit of neurons controlling each different behavioral output regulated by circadian rhythms