The Role of the Circadian Clock in Fat Body Transcriptomics and Metabolomics

URES Presentation | April 22, 2023

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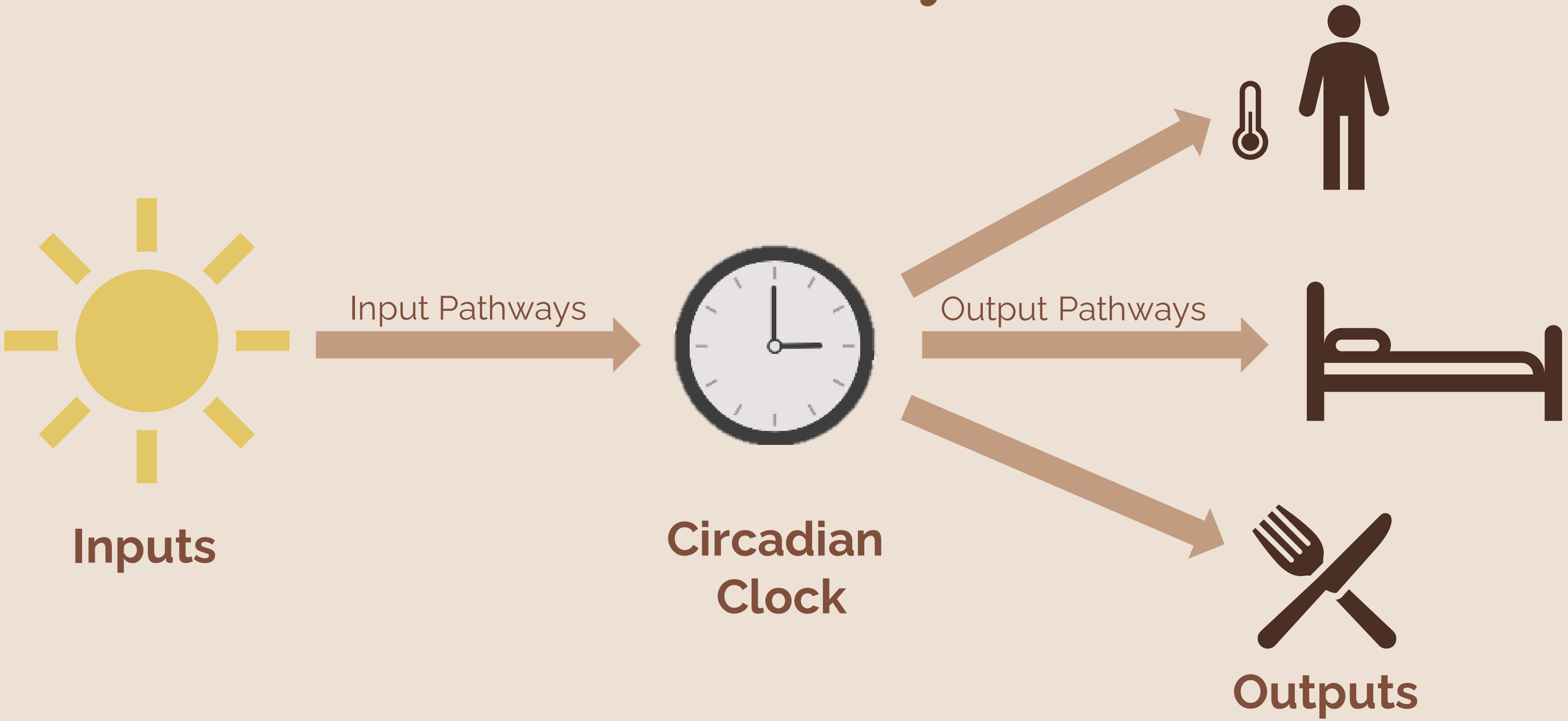
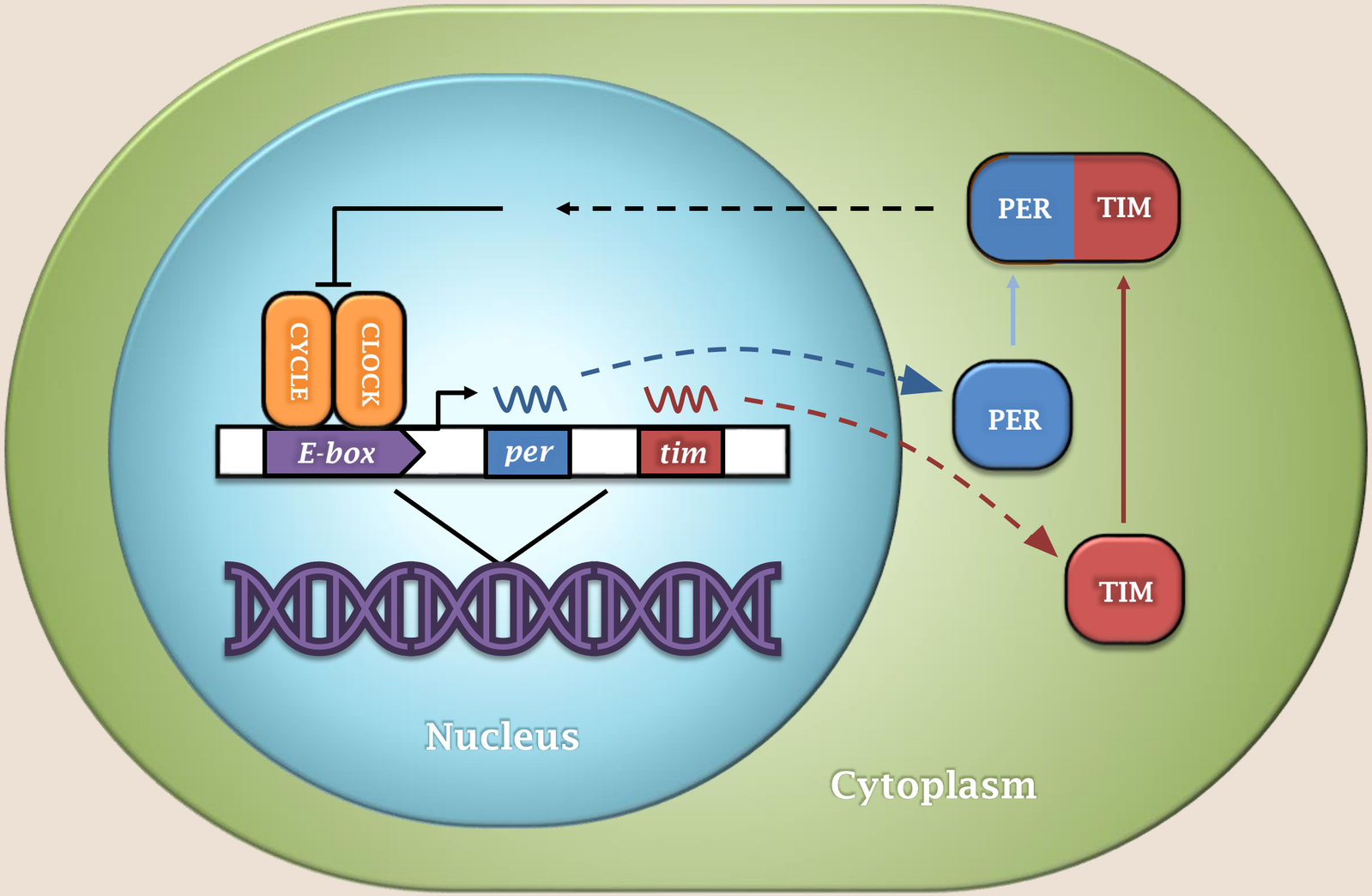
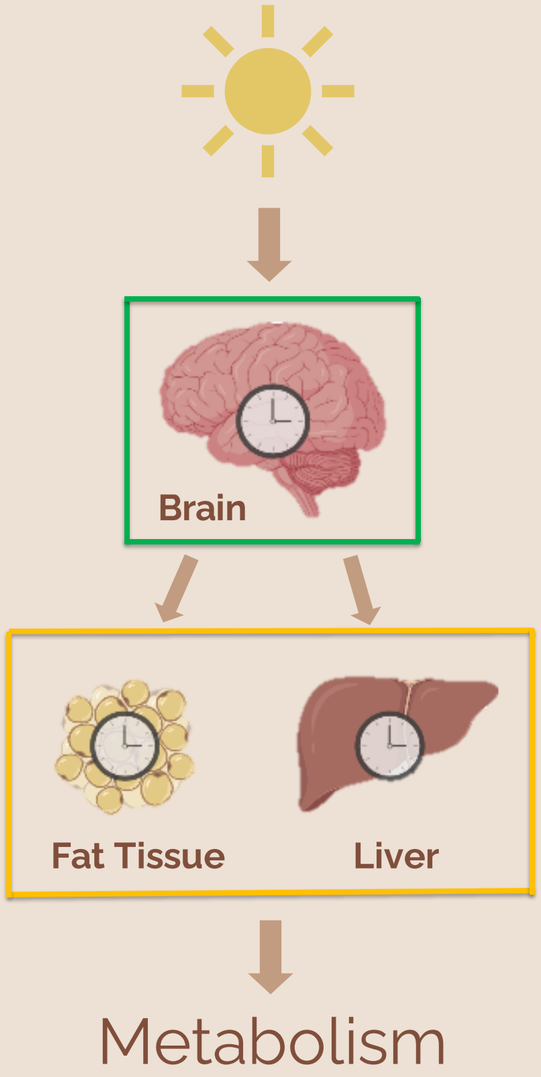
Circadian Rhythms

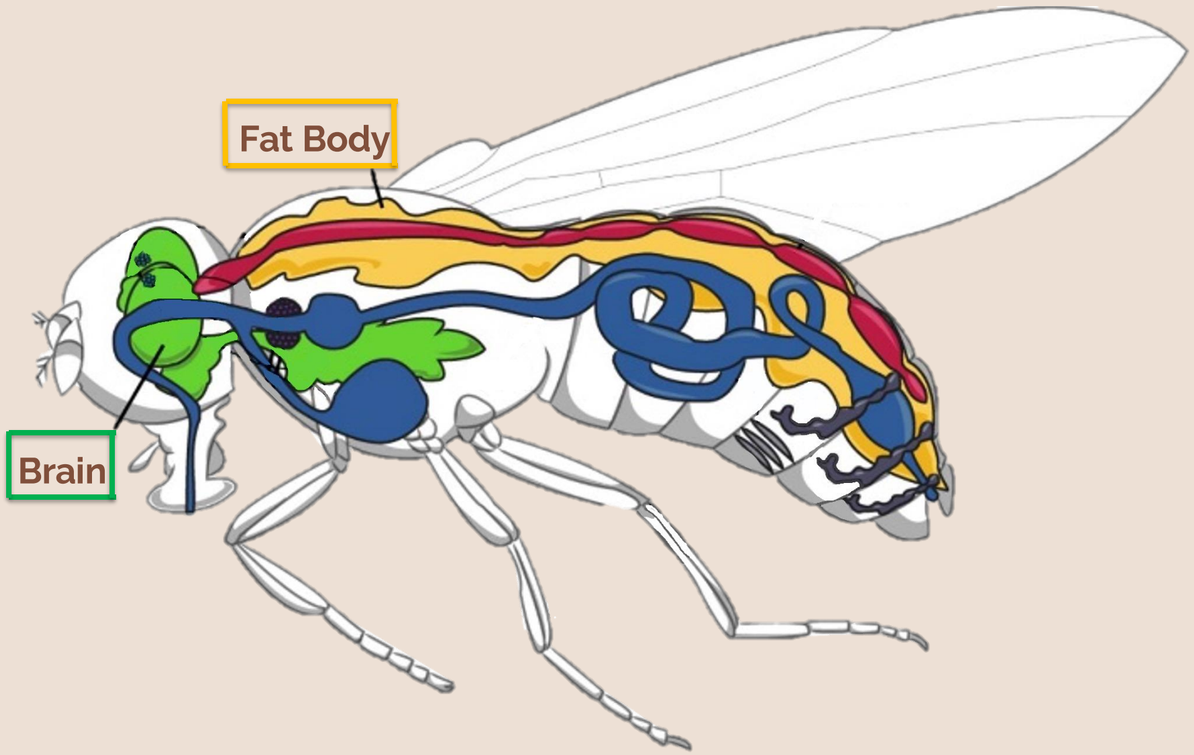


Molecular Clock

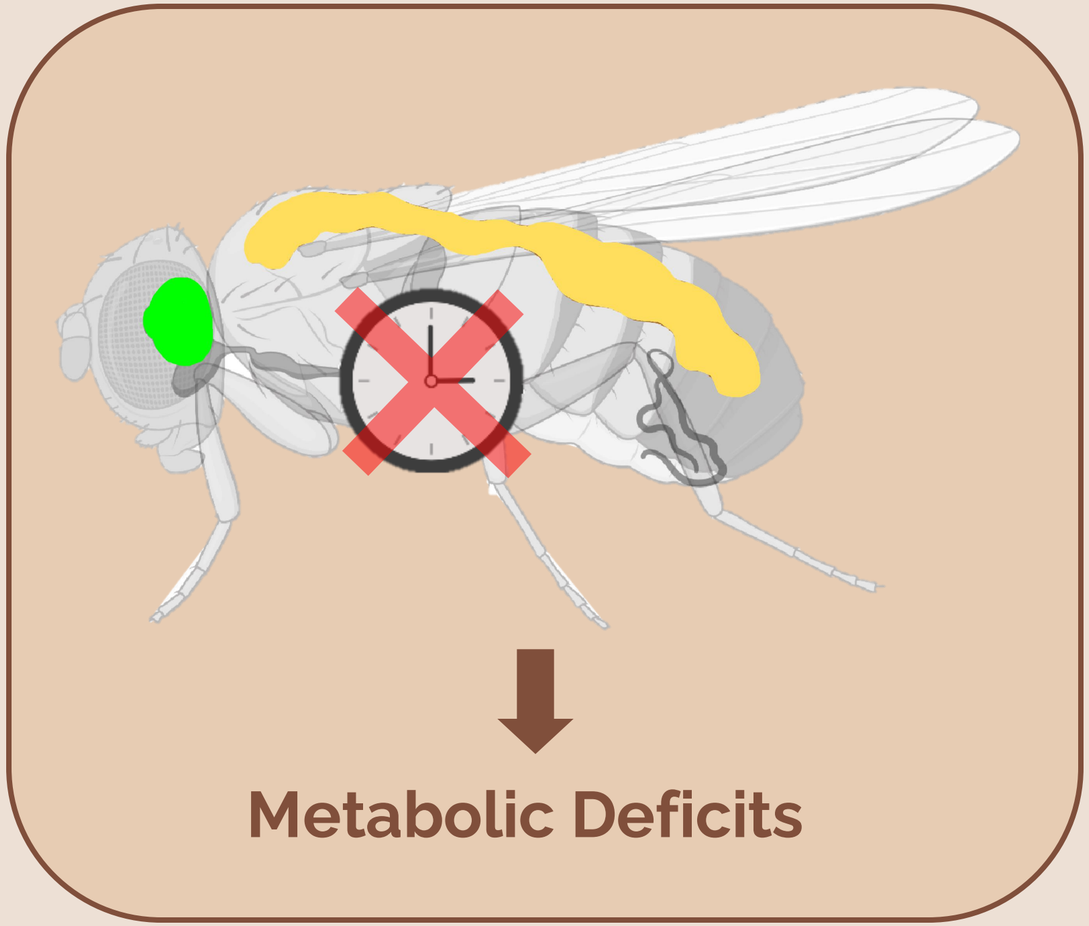


Central and Peripheral Clocks





Major Questions



1. How do central and peripheral clocks regulate metabolism?

2. What is the role of the fat body clock in metabolism?

Experimental Design

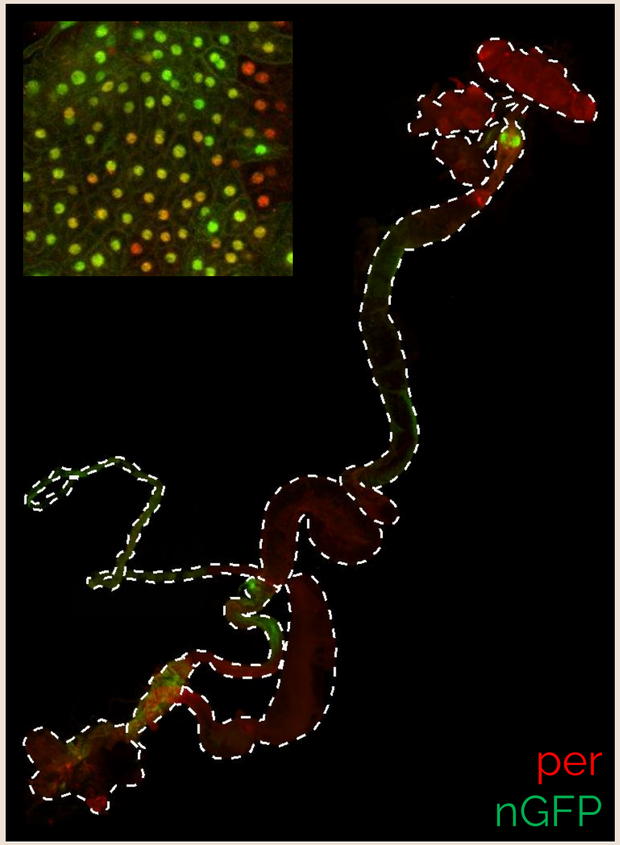
Targeting Brain and Fat Body Clocks

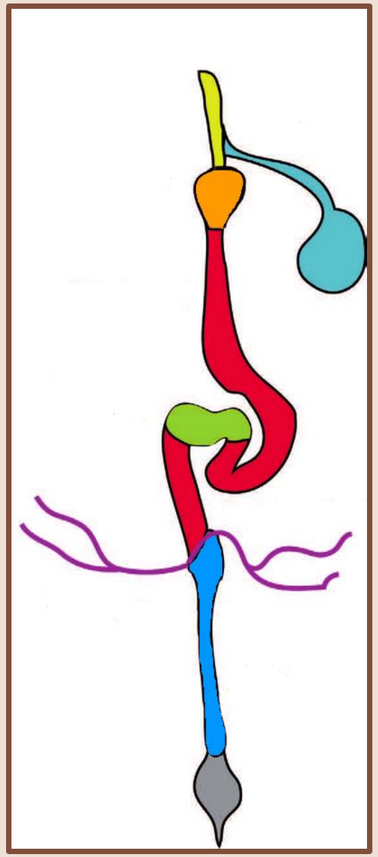
Brain
clk856-GAL4

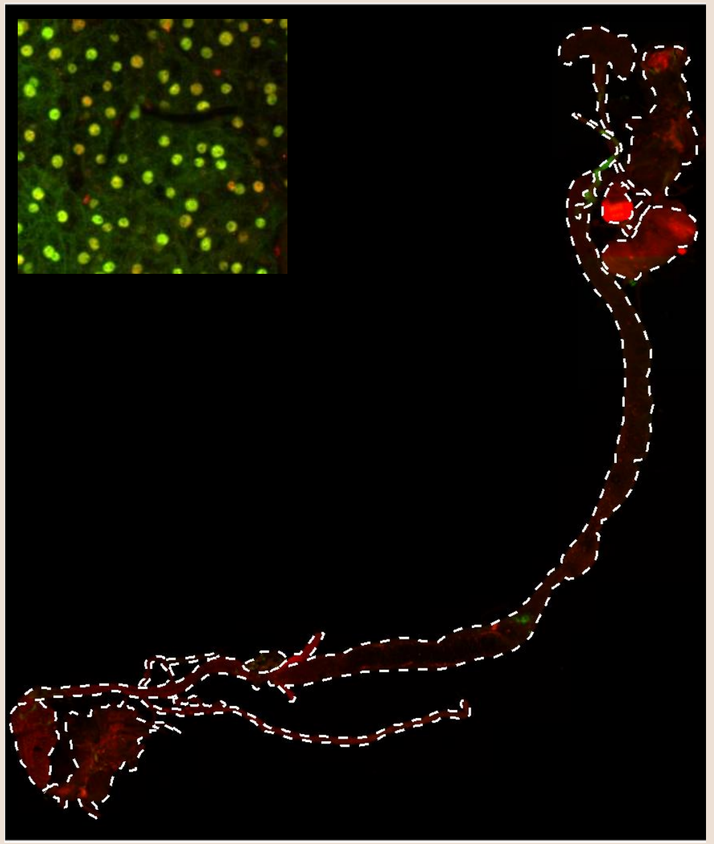
Fat Body
to-GAL4
VS
lsp-GAL4

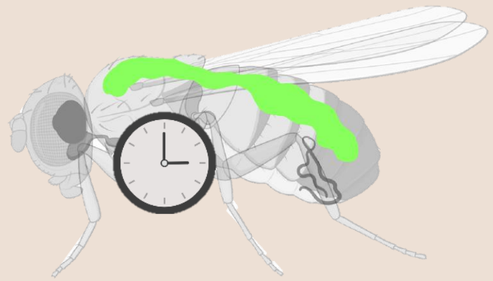
Selectively Targeting Fat Body Clocks: to vs lsp

to-GAL4 vs lsp-GAL4



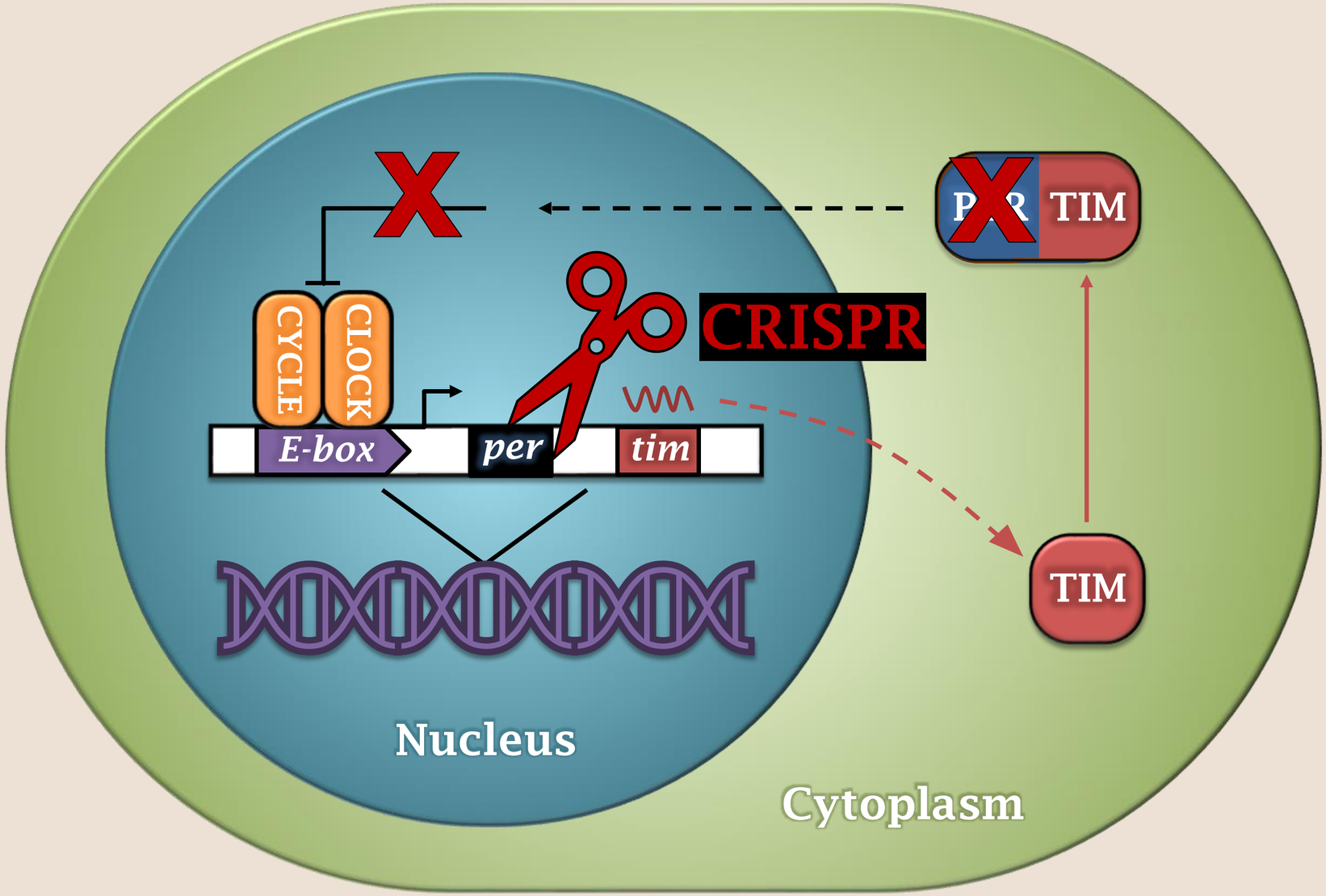






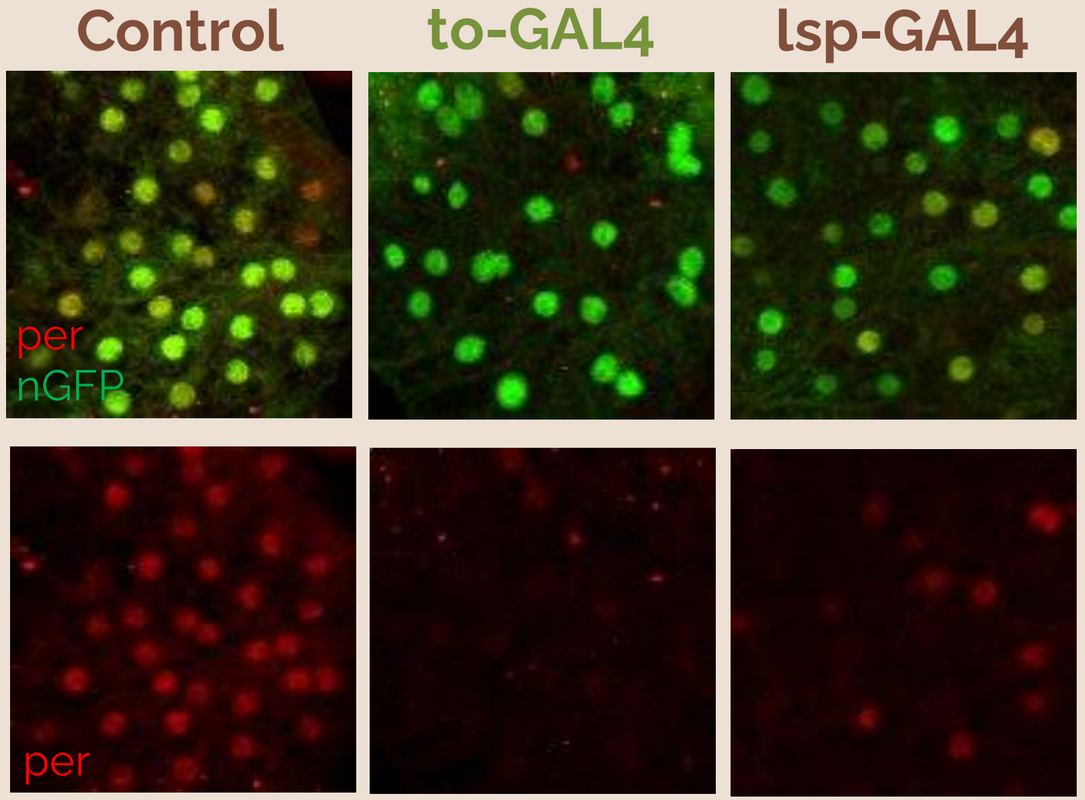
- to- and lsp-GAL4 both selectively target the fat body.
- **lsp-GAL4** is more selective.

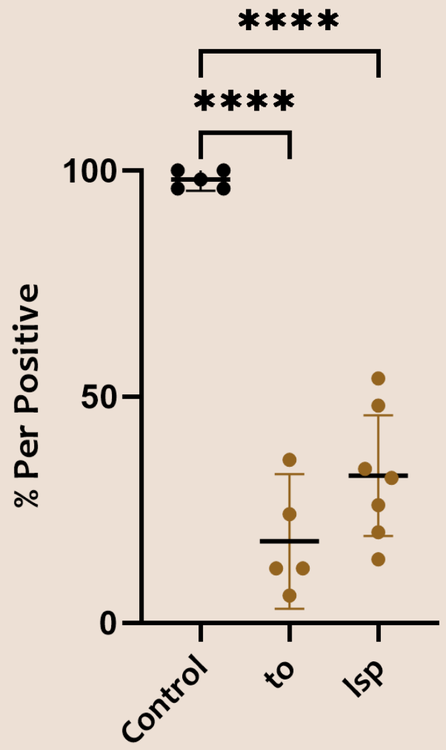
CRISPR-Cas9 Gene Editing



Effectively Disrupting Fat Body Clocks: to vs lsp

CRISPR eliminates clocks most effectively when paired with **to-GAL4.**





Summary:
Targeting and Disrupting Fat Body Clocks

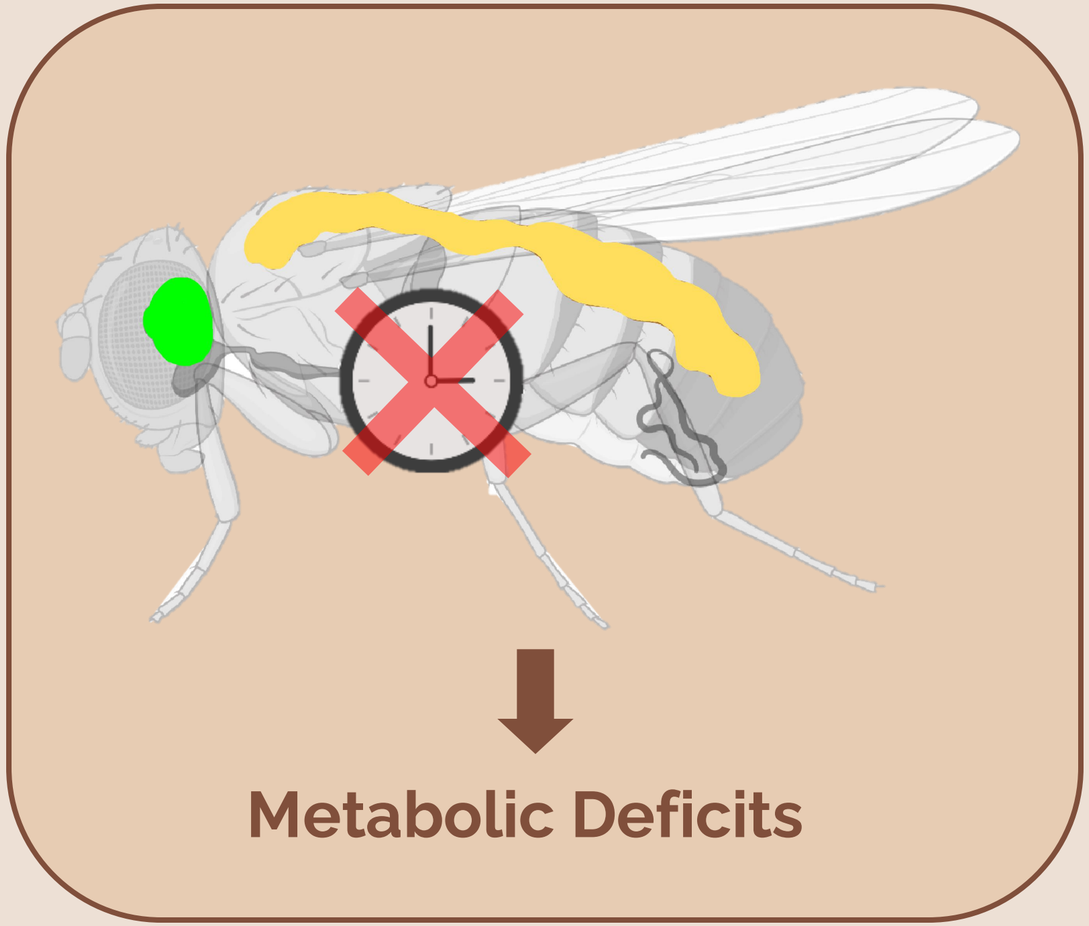
	lsp-GAL4	to-GAL4
Targeting	☑	
Clock Disruption		☑

*to-GAL4 minimally disrupts clocks outside of the fat body.

Conclusion:
We will use **to-GAL4** to drive CRISPR and eliminate PER expression in the fat body.

Experimental Design

Major Questions



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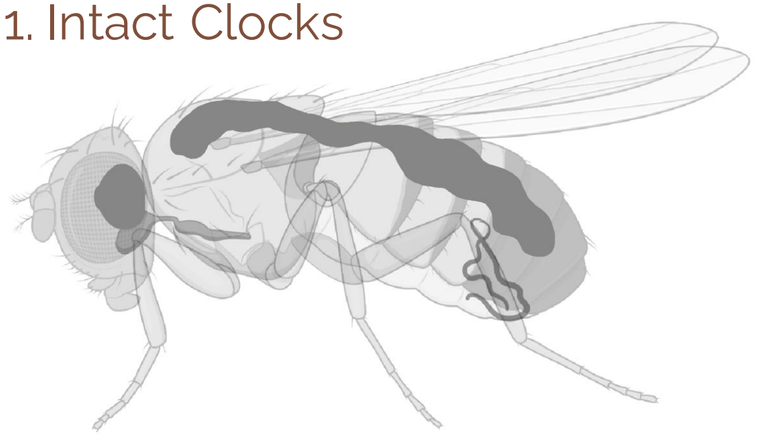
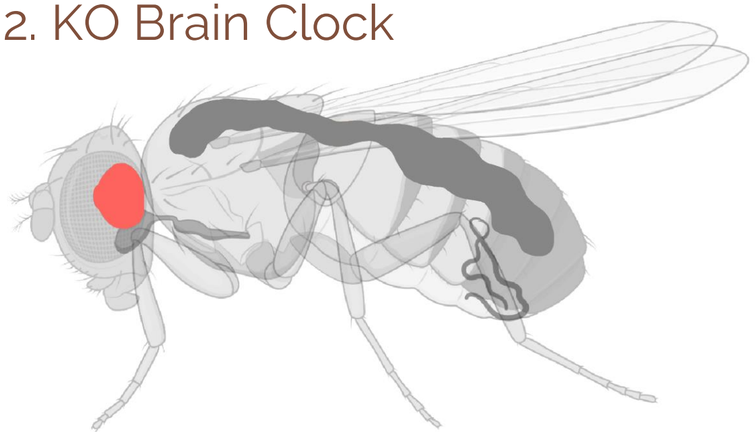
Experimental Groups

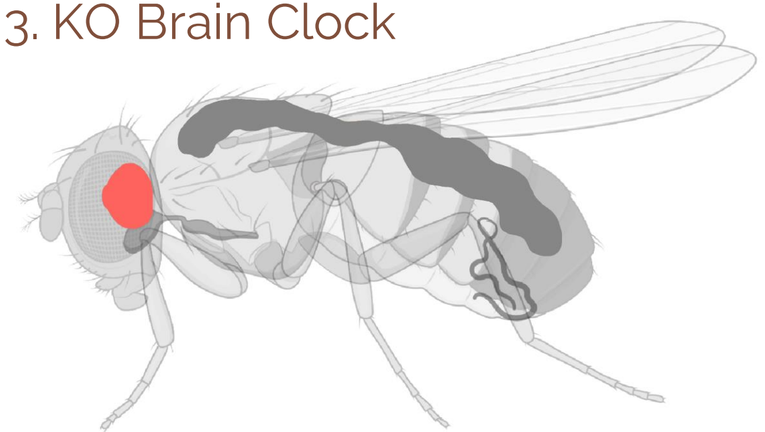
1. Intact Clocks



2. KO Brain Clock

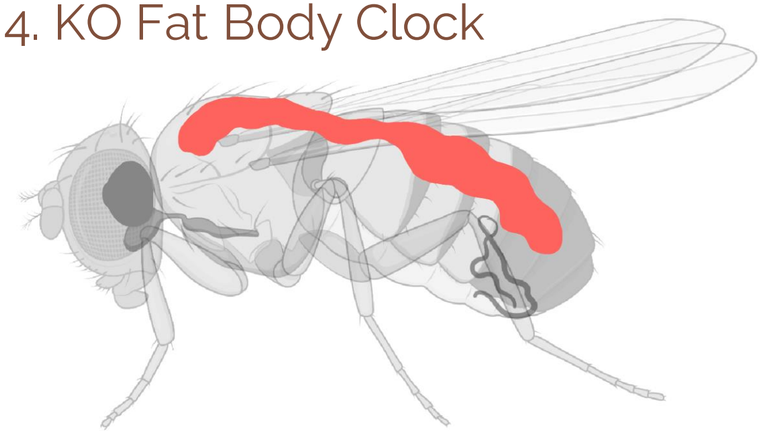


3. KO Brain Clock

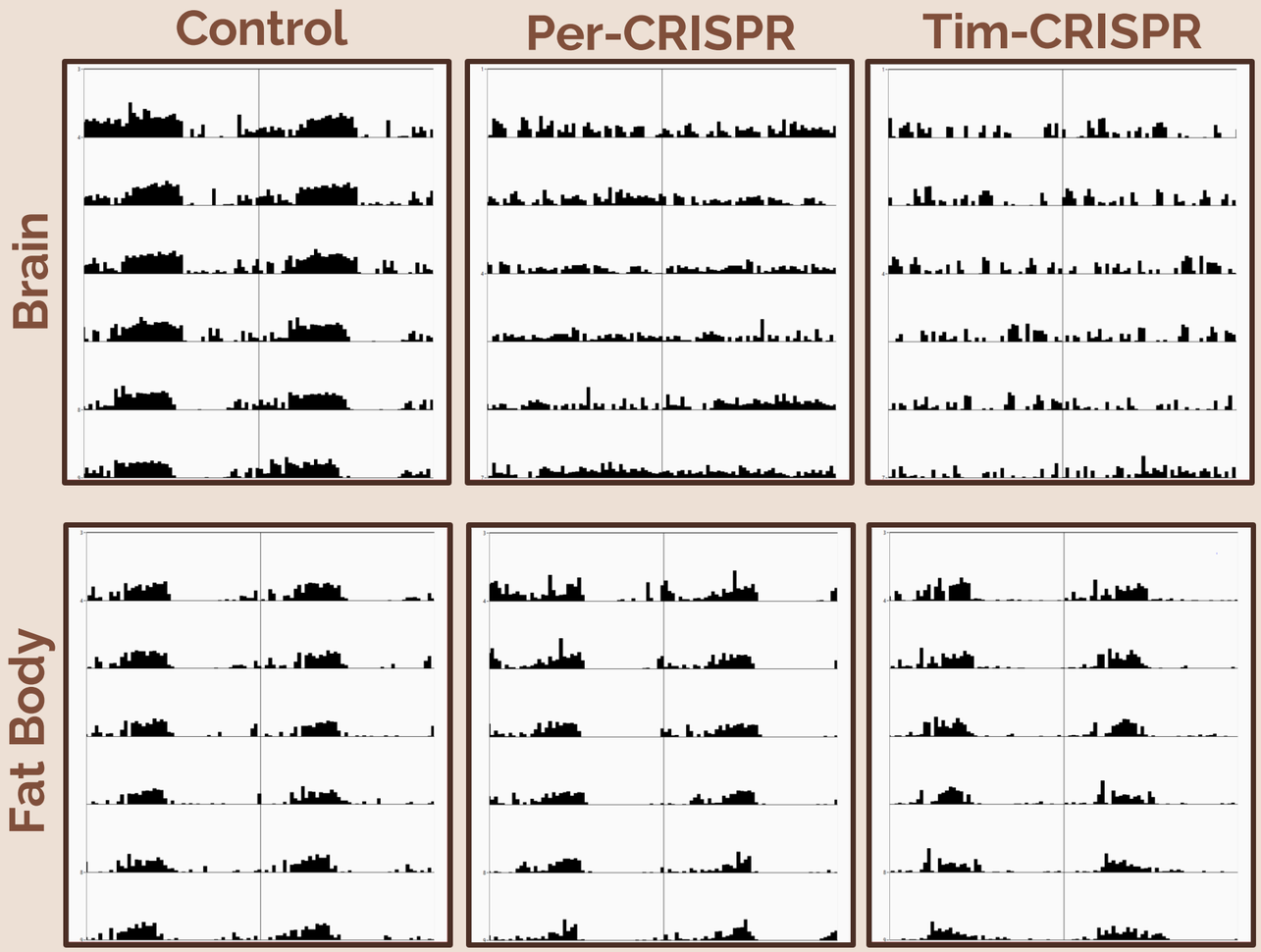


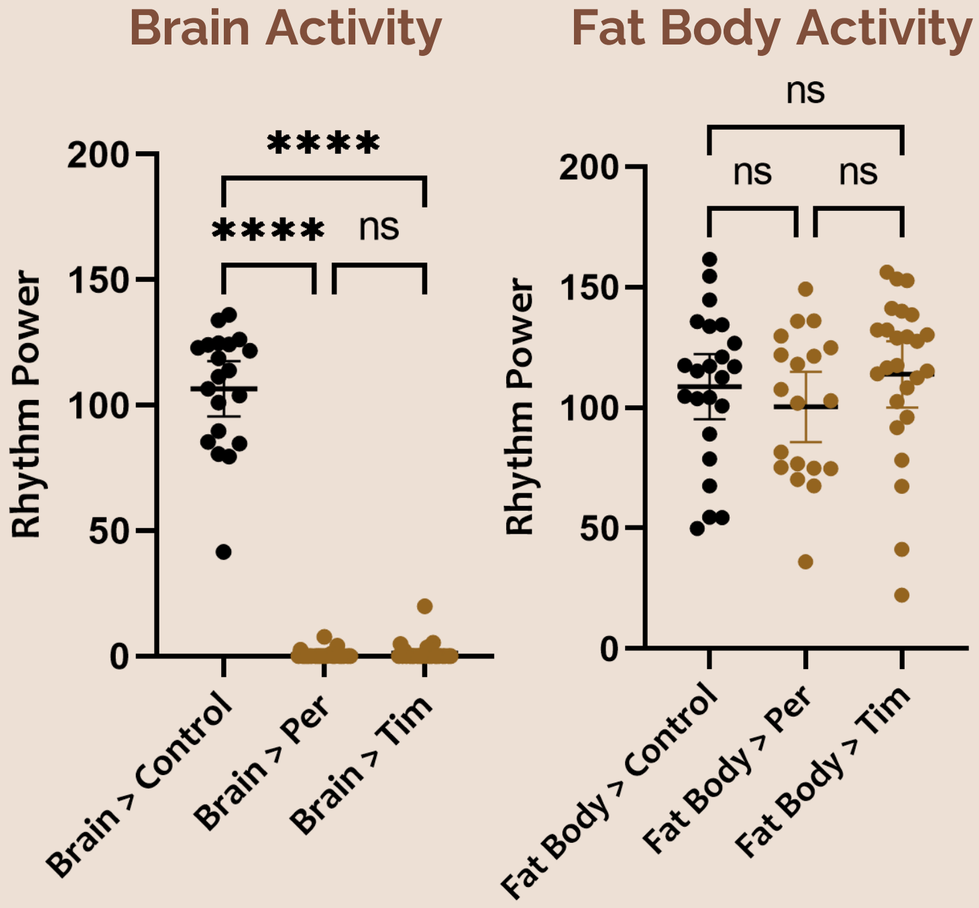
*With Induced Feeding

4. KO Fat Body Clock



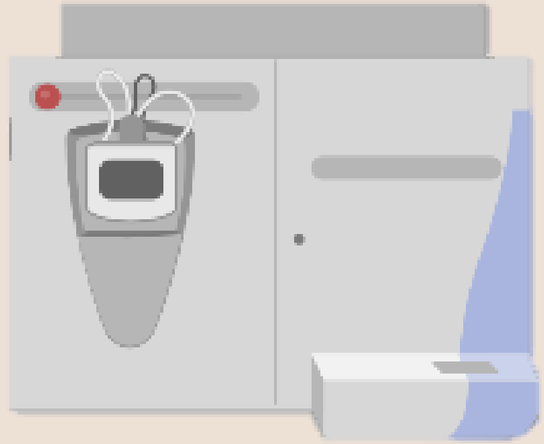
Behavioral Testing





Activity rhythms are under the control of central brain clocks, not fat body clocks.

Metabolomics Workflow



Sample Collection

- Every 2 hours for 48 hours

Data Acquisition

- Mass Spectrometry

Data Analysis

- Identify metabolites

Results: If certain metabolite levels change after clock elimination, their associated metabolic processes are circadian regulated.

Challenges

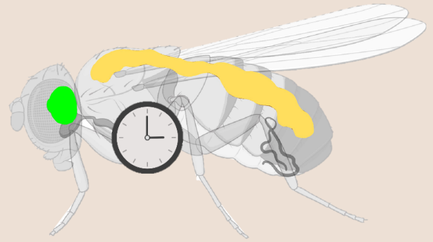
- Number of flies
- Bacterial infection in fly stocks (Summer – Fall, 2022)
  - Required antibiotic treatment in food
- Troubleshooting protocols

$$\frac{24\ collections}{1\ fly\ group} \times \frac{25\ flies}{1\ collection} = \frac{600\ flies}{1\ fly\ group}$$

$$4\ fly\ groups \times \frac{600\ flies}{1\ fly\ group} = 2{,}400\ flies$$

$$2\ replicates \times \frac{2{,}400\ flies}{1\ replicate} = 4{,}800\ flies\ total$$

Conclusions



- to-GAL4 selectively targets and effectively eliminates fat body clock function
- Our metabolomics collection protocol is optimized and scheduled for early summer

Future Directions

- **Metabolomics**: Determine what **metabolites** are impacted by loss of the circadian clock
- **Transcriptomics**:
  - Optimize collection protocol
  - Determine what **genes** are impacted by loss of the circadian clock

Questions?