Wired to Rest - Neural Architecture of Sleep States

Lakshman Abhilash
0000-0002-9933-8989

labhilas@iu.edu

1 Background

Sleep is arguably one of the most ubiquitous, yet mysterious behaviors observed across animal phylogenies. What is sleep? How does one measure it? Do animals other than mammals have sleep-stage-like states, and if so, are the molecular and circuit-level mechanisms that regulate these states known? Are sleep states regulated differently by the circadian clock and by homeostatic processes? And what do we even mean by "circadian" and "homeostatic" regulation in the first place? Do males and females sleep differently, and if so, how is that difference encoded at the molecular or circuit level?

These questions sit at the intersection of ***neuroscience***, ***genetics***, ***animal behavior***, and ***data science***. *Drosophila* has long served as a powerful genetic model for dissecting the functional and circuit-level underpinnings of behavior. A central conceptual bridge, and a central inquiry driving this module, is how fly sleep research can be elevated to meaningfully inform mammalian neuroscience.

2 Experimental Preparation

System and materials.

The primary experimental platform in this module is the Drosophila Activity Monitor (DAM) system, manufactured by Trikinetics Inc. (Waltham, MA).

1. Individual adult flies are loaded into 5mm glass tubes containing sucrose-agar food at one end and sealed at the other with a small piece of white yarn, which permits gas exchange while preventing the flies from escaping.
    i) Sucrose-agar food can be made using the following recipe. For 125mL of food, mix the following in a 1000mL conical flask:
        (a) *125mL de-ionized water.*
        (b) *2.5g Bactoagar.*
        (c) *5g of sucrose.*
    ii) Microwave the mixture until completely dissolved. The solution should be transparent and have a yellow/golden color. Keep an eye to avoid spills.
    iii) Pour the mixture into a glass petri-dish. Make bundles of locomotor tubes with ~35 tubes per bundle. *Note. These tubes must be 65mm long and have a diameter of 5mm.*
    iv) These bundles are then dipped into the glass petri-dish with the media. Food rises up the tubes through capillary action.
2. Each loaded tube is inserted into a DAM monitor, which houses 32 tubes arranged in a grid, with a single infrared (IR) beam passing transversally through each tube.
3. The DAM monitors are then connected to a recording computer as per instructions from Trikinetics Inc..

Experimental logic.

Every time a fly interrupts its beam, the event is registered by the recording computer as a unit of locomotor activity, binned in 1-minute intervals across the full duration of the experiment. This is an elegant example of engineering in service of biology: a highly reliable, automated system capable of resolving the fine temporal structure of behavioral regulation across large numbers of animals simultaneously.

Protocol design.

The experimental protocol spans 14 days and is structured in two phases that together allow circadian and homeostatic regulation of sleep to be dissected independently.

1. Days 1 to 4 (entrainment): Monitors are placed in a controlled environment (an incubator) under a 12:12 Light:Dark (LD) cycle and 25 degrees Celsius. The external light-dark cycle entrains flies' circadian clocks and provides the temporal reference necessary to assess sleep bout timing, phase, and proxies of

sleep depth, analogous to how polysomnography or wearable consumer devices infer sleep-stage architecture from continuous physiological recordings in humans.

2. Days 5 to 14 (free-running): Monitors are transferred to constant darkness (DD). This is done by switching the environment in the incubator without disturbing the monitors. In the absence of environmental timing cues, the free-running, endogenously driven circadian regulation of sleep states can be isolated and quantified without the confound of environmental influence.

This LD-to-DD transition is a foundational paradigm in chronobiology, giving students direct, hands-on experience with one of the most powerful experimental strategies for separating clock-driven from environmentally modulated behavioral outputs in a concrete application of controlled experimental design, as foregrounded in NGSS practices.