Alcohol Susceptibility as a Function of Circadian Rhythmicity in Drosophila and Mammalian Systems: A Review

Ana Pereira, Dr. Lisa C. Lyons

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

Introduction

- Circadian rhythms are referred to as an established pattern of 24-hour physical and behavioral responses regulated by an internal biological clock. The circadian rhythm of an organism, while internally regulated, is entrained by the environment which it is exposed. For both vertebrae and invertebrate animals, biological systems responsible for establishing circadian rhythmicity are most sensitive to light.
- In 2019, the National Survey on Drug Use and Health (NSDUH) reported 14.5 million Americans ages 12 and older having Alcohol Use Disorder (AUD). Chronic consumption of alcohol increases the likelihood of experiencing a stroke, developing chronic heart, liver, and digestive diseases, learning and memory problems, and a disruption of one's circadian cycle.
- To simulate the relationship between alcohol susceptibility and a disrupted circadian rhythm, studies have often used *Drosophila* (typically of the melanogaster specimen) as invertebrate and *Mus musculus* as vertebrate models. These two organisms are particularly utilized as a result of expansive knowledge on how their biological patterns translate to those of humans.

Background

Zeitgeber Time (ZT): standardized unit of time used for entraining a circadian cycle into subjects.

Example: To entrain a 12:12 hour 8 AM - 8 PM light/dark (LD) cycle, ZT = 0 at 8 AM (lights on) and 8 PM (lights out).

Circadian Time (CT): marker of time based off the free-running period of a designated rhythm.

Example: For diurnal organisms, CT = 0 when they become active. Similarly, for nocturnal organisms, CT = 12 when they become active.

Literature Synthesis

	Drosophila	*Mus musculus*
Subject Preparation/ Monitoring Pre-Experimentation	➢ Flies are loaded into and maintained in vials for 1-14 days depending on the desired experimental age. ➢ The standard cornmeal-agar-yeast food media is typically fed to the fly subjects.	➢ Mice are kept in individual cages for 0-4 weeks before the collection of any data. ➢ The feed given is often a nutritionally adequate liquid diet, prepared and replaced daily for subject maintenance.
Circadian Disruption	➢ Genetic: many labs have created their mutants by inducing point mutations (i.e., chemical mutagenesis) or injecting DNA into an embryo to create a transgenic line of flies with mutated genes related to regulating their circadian rhythm: including, but not limited to the *per, tim, cyc,* and *Clk* genes [1]. The mutated flies can then be exposed to constant light or darkness as a means of monitoring their responses without an LD cycle. ➢ Environmental: the baseline or "control" for most circadian related experiments have flies subject to a 12:12 LD cycle in incubators. A random LD shift is a potential mechanism of disrupting sleep cycles [1, 2].	➢ Genetic: mice may be bred or purchased from another research group with mutated genes related to regulating their general internal clock, such as $Clock^{\Delta 19}$ mutants, or that of specific organ mechanisms, such as liver glycogen production in hepatocyte-specific BMAL1 knockout (HBK) mice [6, 5]. Mice may also be mutated to induce a disease not naturally expressed by the specimen, such as targeting polyposis to the terminal ileum and colon of TS4Cre × APC^{lox468} mice as a means of modulating colorectal cancer (CRC) [4]. ➢ Environmental: essentially identical to Drosophila method, except experimental mice experienced 2-3 months of weekly random LD shifts before collection of data [4, 6].
Alcohol Administration	➢ Flies are exposed to EtOH vapor for 15-60 minutes at concentrations between 35-50%. ➢ The vapor may be circulated in vials using a pressurized system or through infusing vial caps with concentrated EtOH for the length of the experiment [2, 3, 1].	➢ Chronic alcohol consumption was simulated via the the Lieber-DiCarli or Nanji liquid diet protocol, containing 15-25% of liquid EtOH in their daily meals for 8-10 weeks prior to data analysis [4, 5, 6].
Common Assays	➢ In vivo: sedation, tolerance, loss-of-righting-reflex (LoRR), and/or recovery testing can be monitored with active monitoring, camera captures, or IR laser beam detection. ➢ Ex vivo: analysis of EtOH content within flies.	➢ In vivo: analysis of excretions to measure concentrations of macromolecules via chromatography [6]. ➢ Ex vivo: staining of organ tissue with organ analysis for the presence of proteins or analysis of polyps (tumors) if studying carcinogenic responses [5, 4].

Discussion

- Research using Drosophila models predominantly track and analyze data collected in vivo. While these fly species may be genetically mutated to destabilize their circadian cycles, the analysis of their data is often centered around behavioral rather than biochemical responses.
- Mammalian models allow for longer term experiments and the investigation of how specific organs respond to a disrupted circadian rhythm along with chronic alcohol consumption.
- Results from studies of either model allow for the pursuit and proposal of solutions to diminish the likeliness of developing debilitating and/or fatal diseases.

References

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