Bioluminescent Monitoring of Circadian Rhythm in Organs-on-a-Chip

Social Acceleration and a Culture of Sleep Deprivation Among College Students

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Ticking Clocks: Understanding the Influence of Circadian Rhythms

What aspects of human life affect and are affected by circadian rhythms?

At the cellular, individual, and societal levels human lives are ruled by patterns. The most dominant of these patterns is the daily alternation between sleep and consciousness, which is a manifestation of innate biological rhythms. These innate rhythms, which recur in a twenty-four-hour cycle and thus termed “circadian”, are important yet poorly understood elements of human health which operate under various levels of synchronization within the body’s cells, organs, and nervous system. While human circadian rhythm follows an approximately daily cycle, the specifics of that cycle are synchronized by *zeitgebers*—external cues which act as "time-givers” for the body’s internal clock (Voigt et al., 2016). Some of the most important zeitgebers are sleep cycles, external light/darkness, and time of eating.

The exact influence and role of circadian rhythms, particularly in individual organs and at the molecular level, is still poorly understood, but it has been established that severe circadian rhythm disruption is associated with detrimental health outcomes such as increased risk for cardiovascular disease and metabolic syndrome (Gagliano et al., 2021). These disruptions are driven by forces inside and outside of the human body: societal pressures such as shift work and social jet lag exist alongside physiological factors like digestion and microbial activity, so analysis of both biological and sociocultural influences must occur for the circadian rhythms to be fully understood.

The proposed research paper seeks to examine the sociotechnical factors which have normalized sleep deprivation and circadian rhythm disruption in the lives of university students. While this STS topic examines how circadian rhythms are influenced at the societal level, the associated technical project is intended to improve the clinical study and understanding of circadian rhythms on the cellular scale via the design of an advanced organ-on-a-chip system with integrated bioluminescence analysis which will allow for real-time monitoring of circadian rhythms in human tissues and organoids.

Bioluminescent Monitoring of Circadian Rhythm in Organ-on-a-Chip Systems

How can organs-on-a-chip be improved to allow for on-chip analysis?

Discussions of circadian rhythm primarily focus on its functions as the human body's internal clock, which is controlled by the central nervous system. However, circadian rhythms are also known to play a role in the function of organs and individual tissues, and investigation of how those mechanisms influence human health is becoming a topic of research interest (Cassone et al., 2017). While the existence of various circadian rhythms is well-known, identifying and examining them is scientifically difficult due to their inherently dynamic and time-dependent nature, for which there are few suitable techniques.

One particular method induces bioluminescence in human cell cultures in order to quantify the occurrence of circadian rhythms living tissues at the molecular level. By adding *luciferase,* a light-producing enzyme derived from fireflies, circadian oscillations result in bioluminescence which can be monitored via a photon-counting luminometer, permitting the study of circadian rhythms on the molecular level (Yoo et al., 2004). This

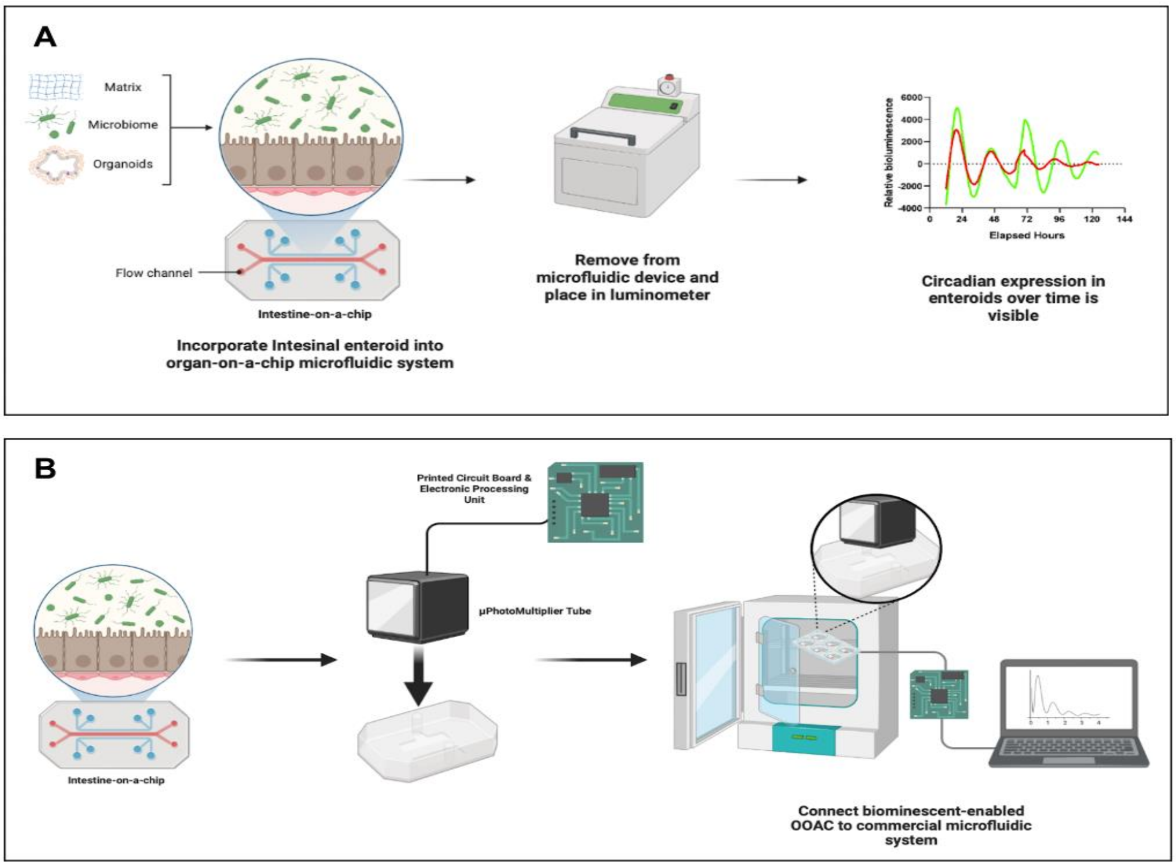

Figure 1 - Method for monitoring of bioluminescence. **(A)** Mouse intestinal organoids are integrated into an OOAC microfluidic device, and circadian rhythms are observed through measurements of bioluminescence via the luminometer. **(B)** Proposed *in situ* setup. A pre-existing Emulate or Mimetas organ-on-a-chip has the micro photomultiplier tube connected on top of the system, which is powered by the printed circuit board and electron processing unit. The bioluminescent-enabled system is placed inside of an incubator to track the enteroids' oscillations over time.

method of researching circadian rhythms is promising, but the usefulness of potential findings is constrained by the accuracy limitations of standard two- and three-dimensional cell cultures. Thus, combining bioluminescent monitoring with improved physiological modelling is a major area of interest for circadian rhythm research.

Organs-on-a-chip (OOAC) are an emerging modeling technology which recreate the dynamic forces and activities of the human body, emulating organ physiology in a complex and realistic manner that surpasses conventional *in vitro* models in both accuracy and complexity. These microfluidic human cell culture devices allow researchers and clinicians to strictly control elements such as fluid flow, tissue microarchitecture, and artificial blood supply. For example, the intestine-on-a-chip system which inspired this project contains: 1) the different epithelial cell types which comprise the lining of the human gut, 2) adjustable nutrient flow, 3) an artificial blood supply, and 4) mechanical stretch to induce structural features in the OOAC that mimic those found in living gut tissue. These key features allow for more robust modeling , which accelerates the testing and development of new medical treatments while also reducing the need for laboratory animals.

Existing OOAC systems, however, lack certain functionalities due to compatibility issues with conventional analysis techniques, which is a significant roadblock for the entire OOAC field (Moreira Teixeira & Mezzanotte, 2021). With current technologies, the cells cultured within organ-chips must be removed before bioluminescence analysis can occur, disrupting the experimental environment and creating non-physiologic conditions that decrease the accuracy of measurements of circadian rhythm expressions.

This research project aims to integrate real-time, *in-situ* bioluminescence monitoring with organ-on-a-chip systems, creating an advanced hybrid instrument. By enabling on-chip bioluminescence analysis, this device will provide a cost-, time-, and labor-effective system that can be used to improve physiological modeling and clinical research capabilities, with vast potential applications to medical and translational discovery.

The primary objective of this technical project is the physical construction of a prototype for the integrated OOAC system. This will involve mounting a bioluminescence-monitoring photomultiplier tube onto a premade organ-on-a-chip and connecting the system to an electronic processing unit. Each stage of this physical integration will require electronic construction and subsequent testing to verify that each of the components retains its functionality. Once the system has been built, it will be tested via experiments for the bioluminescent detection of circadian rhythms in intestinal organoids. Four separate sets of tests will be performed, using either human or mouse tissue cultured on either Emulate or Mimetas branded OOAC systems. Photon-count results from the constructed prototype will be compared to data collected from off-chip analysis using a conventional luminometer.

The prototype device created must measure bioluminescence exhibited by intestinal OOAC tissue cultures accurately and in real-time without requiring samples to be removed from the chip for analysis. Ideally, the device will also be able to collect data from up to twelve separate chips simultaneously and be continuously operational over a period of days to weeks.

By enabling non-invasive analysis of OOAC systems, these capabilities will improve the efficiency and accuracy of clinical research into circadian rhythms as well as other physiological systems and oscillations, potentially leading to new breakthroughs in fields such as disease treatment and diagnosis.

Social Acceleration and a Culture of Sleep Deprivation Among College Students

How have the sociotechnical factors associated with social acceleration influenced the normalization of sleep deprivation among students?

The synchronization of circadian rhythms is an important factor in physiological wellbeing, and appropriate sleep is crucial to maintaining healthy circadian rhythms. While sleep is an innate biological process, it is also socially constructed and elements of sleep vary according to cultural norms, societal demands, and technological influences. The World Health Organization has claimed that there is currently a “global epidemic of sleepiness”, with a decline in sleep duration particularly visible among adolescents and young adults (Lyon, 2019), but the reasons behind this decline have not been comprehensively established.

By examining students’ sleep through the framework of social acceleration, this research seeks to understand how the technological and social developments of modern society have influenced cultural norms around circadian rhythm and lead to the creation of a culture where student sleep deprivation is considered to be par-for-the-course.

Long-term desynchronization between physiological and behavioral rhythms, such as chronic sleep disruption, has been linked to disorders ranging from cardiovascular disease to cancer (Gagliano et al., 2021) and it has been noted that around 70% of college students obtain less than the recommended eight hours of sleep each night, with 60% of students reporting that they are tired or sleepy at least three days a week (Hershner & Chervin, 2014). This reflects a dangerous culture on college campuses, where acute sleep deprivation is so pervasive it has become its own colloquialism: pulling an all-nighter.

Biomedical and Sociocultural Understandings of Sleep

Most modern studies on sleep deprivation and other circadian rhythm disruptions view the problem from a biological or medical standpoint. As previously noted, poor sleep is certainly a medical issue as circadian misalignment has overwhelmingly been associated with negative health outcomes like an increased risk of both physical, such as diabetes and cancer, and psychiatric disorders, such as depression and schizophrenia (Baron & Reid, 2014).

Sociological analysis of sleep (and the lack thereof) is a developing area of research, with discourse emerging on this reciprocal intersection of medicine, culture, society, and the self. One useful framework is social acceleration theory, which argues that key social and technological aspects of contemporary life are speeding up, creating an accelerative trend that effects both cultural and social structures as well as the temporality of individual existences (Hsu, 2014). Sleep is an inherently embodied phenomenon and thus both affects and is affected by social acceleration, and universities are a unique microcosm of broader society. This framework is supported by analyses which have demonstrated that sleep is strongly modulated by social factors (Cacioppo et al., 2002). Application of the concepts associated with social acceleration is thus a relevant framework through which to understand the sleep habits of college students.

At its broadest, social acceleration theory posits that Western society is accelerating in three distinct dimensions: technological development, social change, and the pace of life (Rosa & Trejo-Mathys, 2015). An accelerating pace of life can also be understood as a scarcity of time as a resource, and Rosa describes shortening sleep duration as one consequence of this phenomenon. The coupling of technological advancement and time scarcity creates a "time-pressure paradox": the disjunction between the cultural allure of speed and the common experience of always feeling rushed as interrogated by Judy Wajcman (2014). The framework of social acceleration thus describes time as intrinsically both social and personal; cultural norms and social pressures couple with technological reality to produce temporal experiences with individual material consequences.

Numerous scientific studies have established widespread sleeplessness among students, and it is important to examine how modern technology has contributed to this normalization. One example is how the ubiquity of smartphones and remote learning technologies contribute to telepressure, an always-connected mentality which has been associated with increased stress and decreased sleep among students (Barber & Santuzzi, 2017). Smartphones and technology are also involved in other sleep-damaging behaviors such as doomscrolling, bedtime procrastination, and late-night blue light exposure. The habits and pressures associated with electronic devices are thus key elements to consider when examining student sleep habits.

Methods and Analysis

This research project aims to use social acceleration theory to examine in-depth the factors which have normalized poor sleep practices among college, with supporting evidence gathered through ethnography and literature analysis. The ethnography will involve interviewing current students at the University of Virginia about their sleep habits, personal attitudes around sleep, the factors which they think impact their sleep, and how their peer's sleep habits and attitudes. This ethnographic analysis will provide qualitative information about the pressures affecting and normalizing sleep deprivation among college students.

The literature analysis, performed by reviewing biomedical studies and sociological discourses on sleep as published in academic journals, will then be used to draw connections between those lived experiences, pre-existing sleep studies, and broader social acceleration theory.

Conclusion

Through ethnography, literature review, and utilization of the framework of social acceleration, the proposed sociotechnical analysis aims to examine the pressures which have led to widespread and normalized sleep deprivation among college students. The identification and discussion of these factors will allow for an improved understanding of the sociotechnical influences on circadian rhythms and human health. Additionally, understanding of the biological and molecular impacts on circadian rhythms will be improved by the technical project. The construction of an organ-on-a-chip system with integrated bioluminescence monitoring also has the potential to significantly improve clinical research capabilities. Both the STS and technical research projects will thus contribute to knowledge of the complex network of elements which contribute to and arise from human circadian rhythms. Ideally, these research projects will allow for further work to be done which investigates the interplay between factors influencing circadian rhythm at all levels, from the societal to the molecular, in order to develop a more complete picture of human health.

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