Internship report

12 June 2024- 10 Sept 2024

Kartik Singh

Shivaji College, University of Delhi

SPATT SYSTEMS PHARMACOLOGY AND TRANSLATIONAL THERAPEUTICS

Perelman SCHOOL OF MEDICINE UNIVERSITY of PENNSYLVANIA

Penn
University of Pennsylvania

J-1 Student Intern Evaluation Form

Last updated 4/29/2019

As per the U.S. Department of State regulations, the hosting Penn faculty mentor must evaluate the progress and performance of the J-1 Student Intern prior to the completion of the internship. **All internships require a final concluding evaluation. The internships lasting longer than six months also require at least one additional evaluation undertaken at the midpoint of the program.** The hosting department must retain J-1 Student Intern evaluations (electronic or hard copy) for at least 3 years following the completion of each intern's program.

A copy of each evaluation must be sent to ISSS within 5 business days of the J-1 Student Intern's program end date. *Should the hosting department neglect to provide ISSS with a copy of evaluation(s), the department will be barred from hosting any J-1 Student Intern for 12 months.*

J-1 Student Intern's Name: Singh / Kartik /
(Family/Last) (Given/First) (Middle)

Faculty Mentor's Name: Aalim M Weljie

Faculty Mentor's Title: Associate Professor

Department Name: Systems Pharmacology and Translational Therapeutics

Check One: ☐ Midpoint evaluation ☒ Final evaluation

Evaluate the Student Intern's performance on the tasks outlined in the original DS-7002 Training Plan:

☒ Excellent ☐ Above Average ☐ Average ☐ Below Average

Comments on the Student Intern's performance:

Kartik is hardworking and motivated. He demonstrated efficiency in managing multiple projects and worked with multiple members of the lab. He worked in two major projects - 1. Metabolomics of Drosophila sleep and circadian rhythm and 2. Metabolomics of obstructive sleep apnea. He was involved in planning, exceution, and analysis of results in both projects. Doing so, he proves his ability to work in team. He is also very organized and keen to learn new techniques. Kartik presented his work on a weekly basis in the lab and demonstrated considerable advancement within a short period of time.

How would you rate the overall student internship program and its benefits to your department and Penn?

☒ Excellent ☐ Above Average ☐ Average ☐ Below Average

How this program has benefited Penn and the J-1 Student Intern in terms of educational and cultural exchanges?

Kartik learned several new techniques which are otherwise not taught in regular curricula in India. For example, techniques like fly dissection, sleep assay, mass spectrometry and NMR spectroscopy and their utility in translational science certainly enriched him as a student of science.
This visit was Kartik's first in the US and he had plenty of opportunity for learning about the rich cultural and natural history of the US. He took part in the 4th of July celebrations, visited multiple museums in the Philadelphia, Washington, DC and New York City area.

Faculty Mentor's Signature: [signature] **Date:** Aug 30, 2024

Student Intern's Signature: [signature] **Date:** Aug 30, 2024

Table of contents

Objective 1 –

<u>Understanding role of dietary intervention in mitigating the negative consequences of sleep loss using *Drosophila* short sleeper fly.</u>

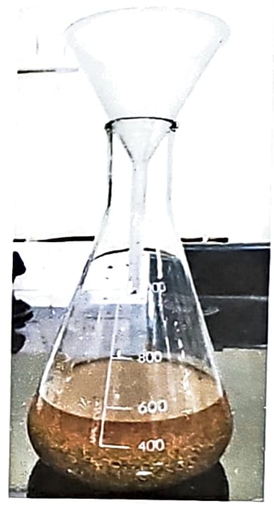
Drosophila melanogaster, or the fruit fly, has a genetic makeup that allows researchers to study the impact of sleep and its regulation. The short sleeper variant has been specifically engineered to exhibit reduced sleep duration, mimicking conditions of sleep deprivation seen in humans. This model enables scientists to observe behavioral, physiological, and molecular changes resulting from sleep loss.

Studies indicate that sleep deprivation can lead to negative consequences such as impaired cognitive function, increased stress levels, and metabolic dysregulation. Dietary interventions, such as altering macronutrient composition or introducing specific supplements, can play a significant role in counteracting these effects. For instance, high-protein diets or specific fatty acids have shown promise in restoring cognitive function and reducing stress in sleep-deprived flies.

Abstract 1 –

Sleep loss is increasingly recognized as a significant public health concern, leading to various negative health outcomes, including cognitive impairment and metabolic dysregulation. This study explores the role of dietary interventions in mitigating the adverse effects of sleep deprivation using the Drosophila short sleeper fly model, which genetically mimics reduced sleep duration. We investigated how different dietary compositions, including variations in macronutrients and specific bioactive compounds, influence behavior, and overall health in sleep-deprived flies. Our results try to find out that targeted dietary interventions can significantly restore cognitive function and improve metabolic markers in short sleeper flies. Notably, low-protein diets and certain fatty acids were not effective in reducing stress levels and enhancing activity. This research highlights the complex interplay between sleep and health, providing a foundation for potential nutritional strategies to alleviate the negative consequences of sleep loss. By leveraging the Drosophila model, we aim to uncover underlying mechanisms and identify therapeutic dietary options that could benefit individuals experiencing sleep deprivation. These findings underscore the importance of integrating dietary considerations into sleep health management, with implications for both basic research and clinical applications.

Materials and instruments used-



Basic *Drosophila* anatomy

An adult fly has two compound eyes and, three-part bodies (head, thorax, and abdomen), wings, and six jointed legs, which are typical of most insects. The varied sorts of bristles and hairs seen on the body will be used to identify distinct phenotypes of flies.

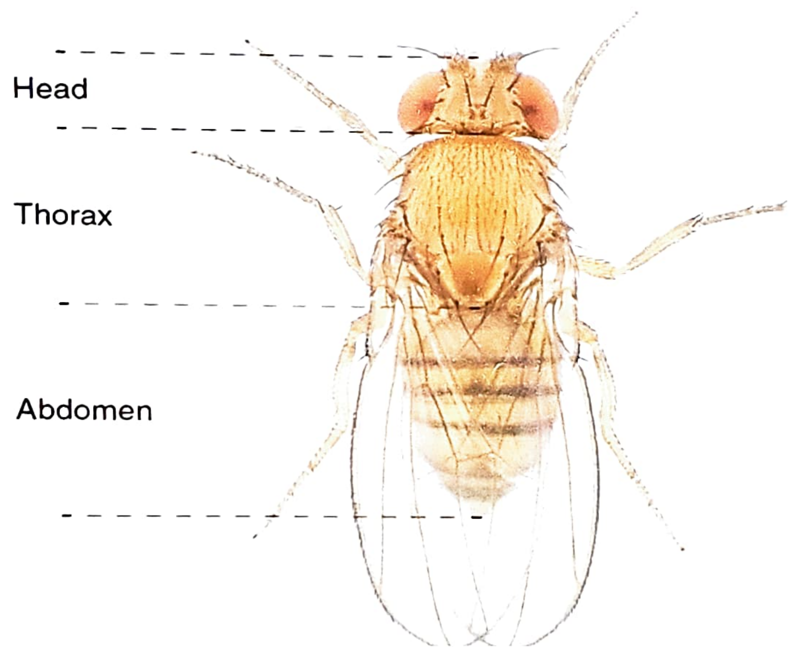


Figure : *Drosophila Melanogaster anatomy.*

Classification of *Drosophila*

Female *Drosophila* are bigger in size and male flies are smaller and have a darker and more rounded abdomen. Virgin females are much larger than older females and have lighter body color compared to mature females. In addition, immediately after eclosion, a visible dark greenish spot (the meconium, the remains of their last meal before pupating) can be seen in virgin flies on the underside of the abdomen.

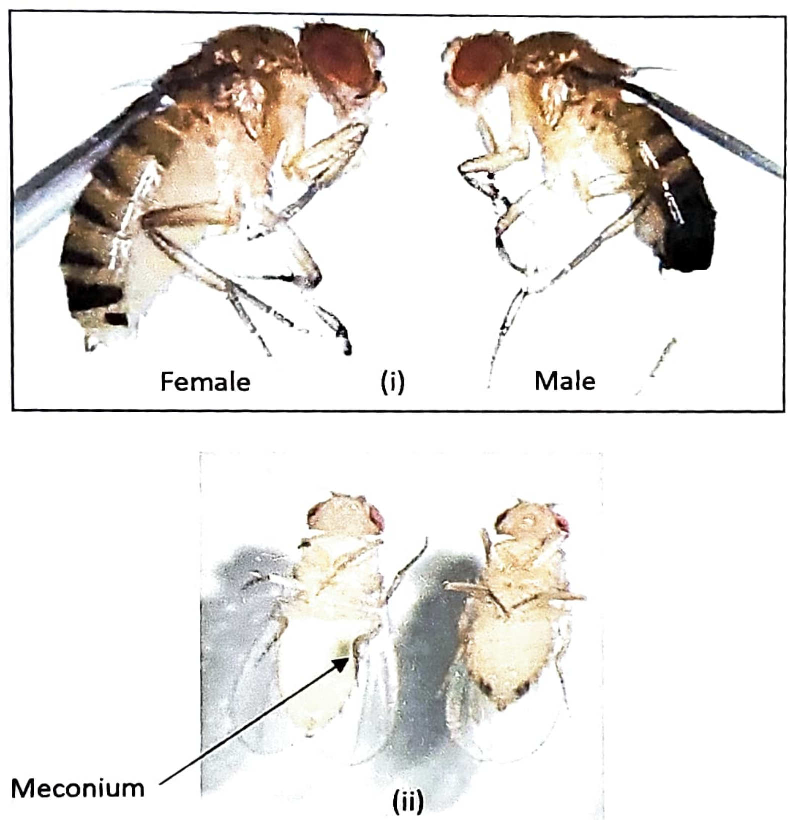


Figure: *(i) Male and Female Drosophila Melanogaster.*
(ii) Identification of a virgin female fly.

Life cycle of *Drosophila*

Female and male flies' mate for several days when maintained in vials or bottles and produce progeny. An adult female lays 50-75 eggs daily (about 450 within 10 days). After 1 day, the egg hatches. After 24 hrs, the 1st instar larva appears of about one day in length. On the third day, 2nd instar larva appear, also about one day in length.

On the fifth day, 3rd and final instar larva appears which is about 2 day in length.

On the seventh day, larva begins roaming, pupariation occurs on the 9th or 10th day. On the 11th or 12th day, adults emerge from the pupal cases ; females become sexually mature 8-10 hours after emergence.

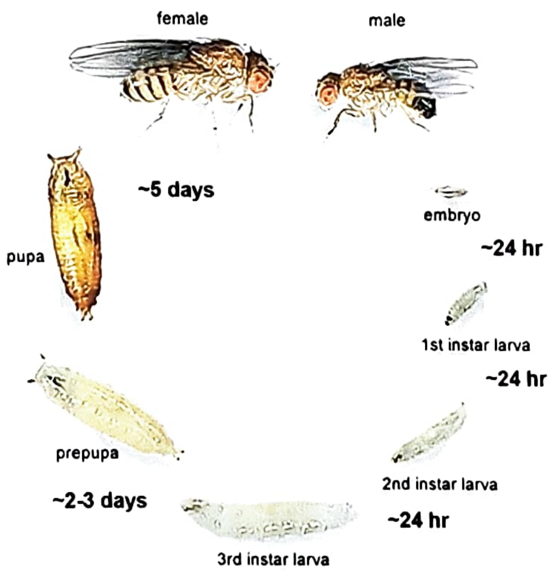


Figure: *Life cycle of Drosophila Melanogaster.*

Benefit of using *Drosophila* as a model system

1. They are small and can be easily handled.

2. They can be easily anesthetized and manipulated individually with unsophisticated equipment.

3. They are sexually dimorphic (males and females are different), making it is quite easy to differentiate the sexes.

4. Virgins fruit flies are physically distinctive from mature adults, making it easy to obtain virgin males and females for genetic crosses.

5. Flies have a short generation time (10-12 days) and do well at room temperature.

6. The care and culture of fruit flies requires little equipment, is low in cost and uses little space even for large cultures.

7. *Drosophila* has four pair of chromosomes carrying all the genes on them.

8. Simple genetic, molecular and behavioral assays are there to easily score the phenotypes.

Drosophila activity monitor system (DAM)

The Drosophila activity monitor is a specialized tool used to study the behavior and activity levels of fruit flies (Drosophila melanogaster) in a controlled environment. This system typically consists of a series of infrared beams or motion sensors placed in a chamber that records the flies' movement. Researchers can analyze patterns of activity, rest, and response to various stimuli, providing insights into genetics, neurobiology, and circadian rhythms.

By tracking individual flies or groups, scientists can observe how different genetic modifications or environmental conditions affect behavior. For example, researchers might investigate how mutations in specific genes influence activity levels or how environmental factors like temperature and light impact circadian rhythms.

Drosophila's relatively short life cycle and well-mapped genome make it an ideal model organism for such studies. The activity monitor allows for high-throughput screening, enabling large-scale experiments that can yield statistically significant results. Overall, this tool is invaluable for advancing our understanding of fundamental biological processes and can have implications for broader research areas, including sleep disorders and neurodegenerative diseases.

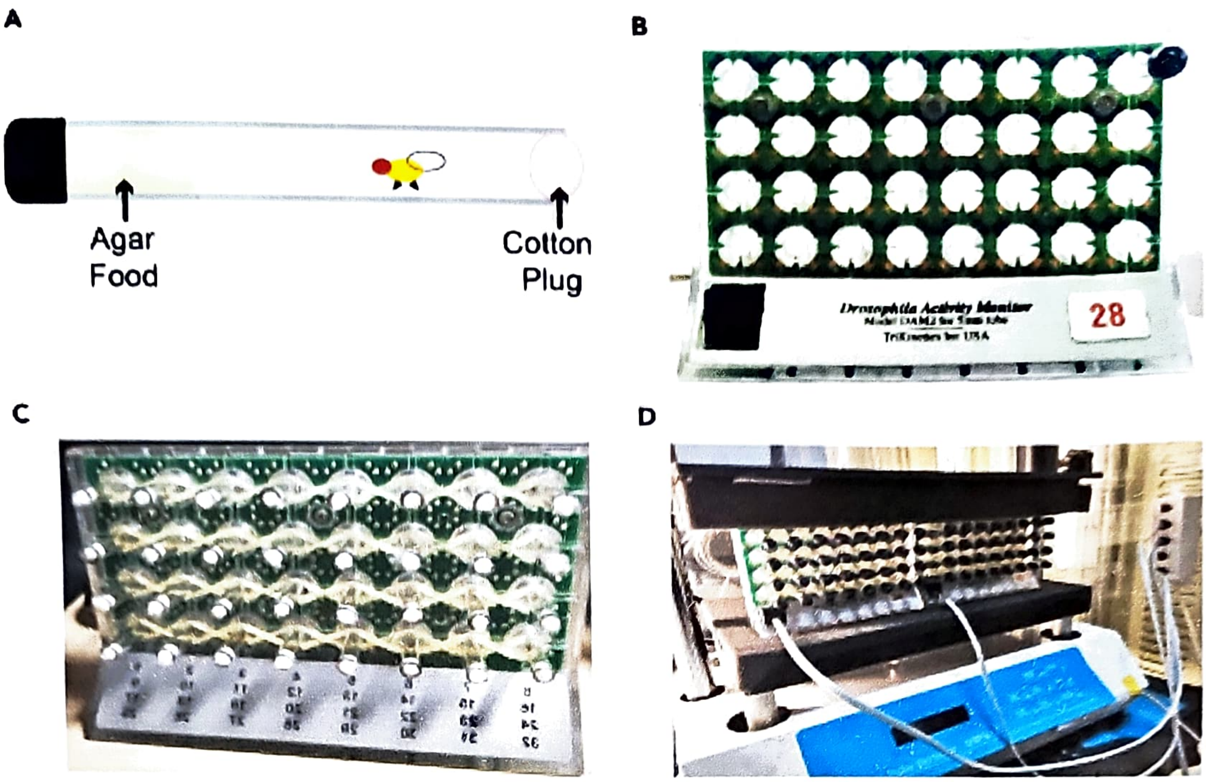


Figure *: Image of Drosophila activity monitor (DAM) system and how it is being used to understand sleep and circadian cycle. Source of image: ScienceDirect*

Why is *Drosophila* good model system to study sleep and circadian cycle.

Drosophila melanogaster, commonly known as the fruit fly, is an excellent model organism for studying sleep, circadian rhythms, and overall homeostasis for several reasons:

1. Genetic Simplicity and Manipulation

- **Well-Characterized Genome**: Drosophila has a relatively simple and well-mapped genome, making it easier to identify and manipulate specific genes related to sleep and circadian rhythms.
- **Genetic Tools**: Researchers can easily create genetic mutants, knockouts, and transgenic lines. This allows for the exploration of specific gene functions and their roles in sleep regulation and circadian cycles.

2. Observable Sleep Behavior

- **Quantifiable Sleep Phenotypes**: Drosophila exhibit clear and measurable sleep-like states, allowing researchers to quantify sleep duration, patterns, and disturbances. Sleep in fruit flies can be observed and measured using activity monitors, which track movement and inactivity.
- **Response to Stimuli**: The effects of various stimuli (e.g., light, temperature, and drugs) on sleep can be easily tested, providing insights into the environmental factors influencing sleep and circadian rhythms.

3. Homeostatic Regulation

- **Energy Homeostasis**: Drosophila are useful for studying the interplay between sleep and metabolic processes. Researchers can examine how sleep deprivation affects energy balance, feeding behavior, and metabolism, which are critical for understanding overall homeostasis.
- **Stress Response**: The fruit fly's response to various stressors (e.g., oxidative stress, heat, and nutrient availability) can help elucidate how sleep and circadian rhythms contribute to maintaining homeostasis in living beings.

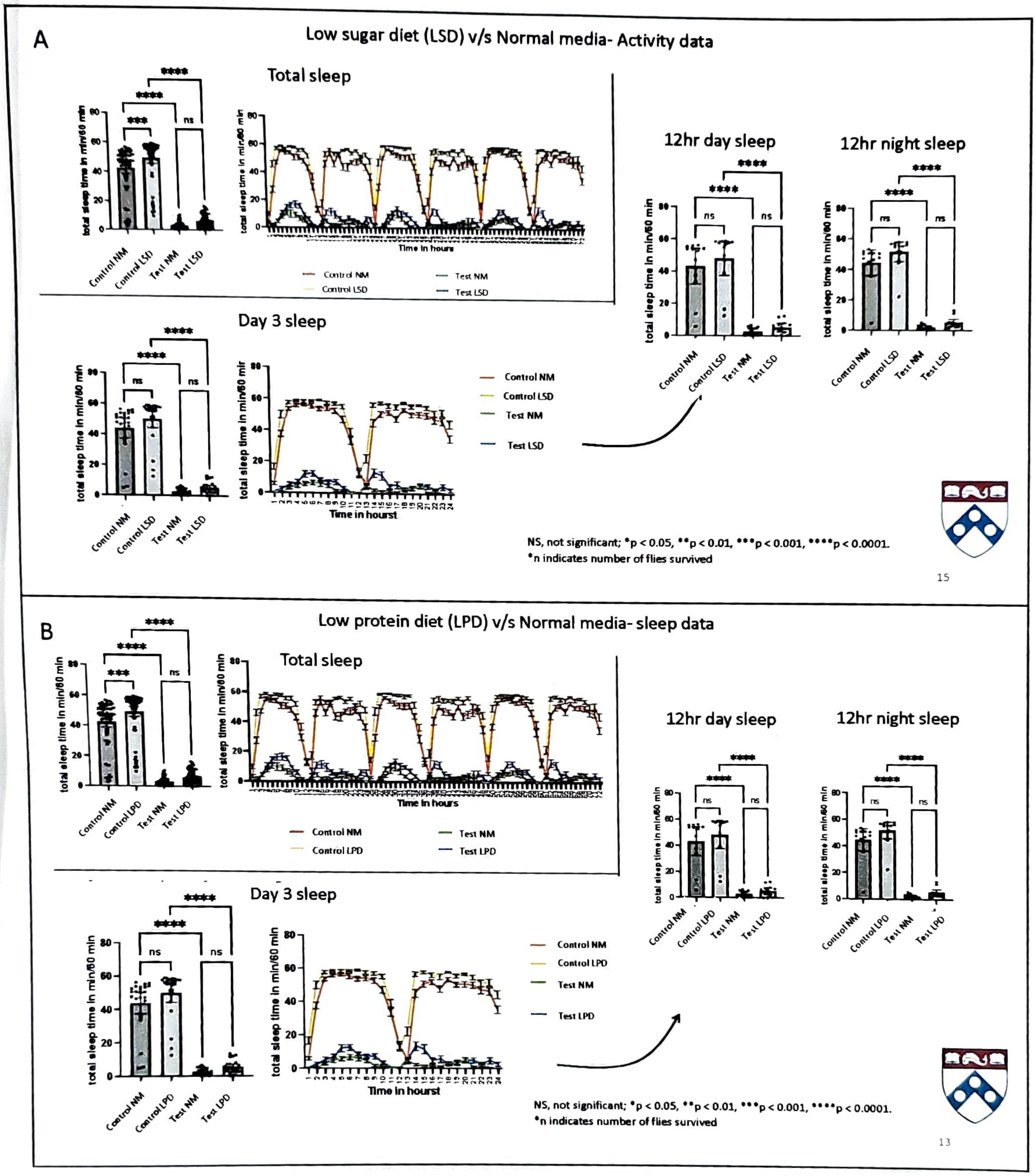
DAM monitor preparation —

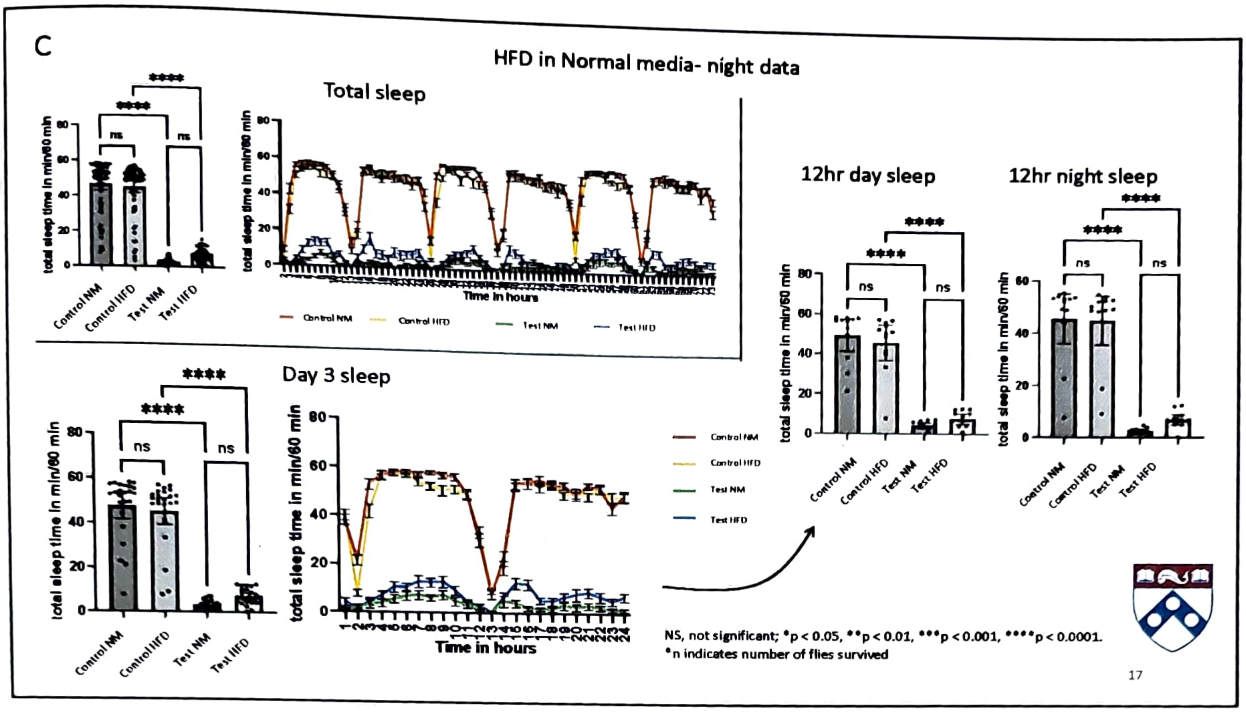
The flies were fed on the respected media of interest for the experiment, for about 4-5 days after being 2-3 days old. After feeding upon the media, 32 male flies (both control and mutant) were set up on single beam drosophila activity monitor and were set in an 12-12 LD incubator for sleep and activity assay.

The media conditions weren't changed, as the objective was to see the effect of different types of diets as course of their life.

Results 1 —

Night activity to see if different diets induce sleep --





***Figures:** these figures correspond different types of diets tried with control and mutants, and the graphs shows the sleep data on the span of 3 days. Each box has a different medium being fed to the flies; and during the sleep assay, the flies were being fed the same media. The box has activity for 3 days, and the activity of one day was chosen and split to see if there is any day or night sleep difference between the controls and short sleeper mutants.*

Discussion-

The investigation into the effects of various diets on short sleeper mutants has revealed intriguing insights regarding sleep and activity patterns in Drosophila. The findings suggest that none of the dietary interventions tested effectively induced sleep in these mutant flies, which is significant given the correlation between sleep and activity levels.

Diets Tested on Short Sleeper Mutants

1. **Low Sugar Diet (LSD)**:
   - As illustrated in **Figure A**, the data shows a statistical analysis indicating that the low sugar diet does not promote sleep in the short sleeper mutants. The lack of sleep induction corresponds with minimal changes in the activity levels of the flies. Both the control group and the mutants exhibit similar activity patterns, suggesting that the low sugar content does not influence their overall behavior or induce a sleep state.
2. **Low Protein Diet (LPD)**:
   - **Figure B** presents data from the low protein diet, which further supports the previous findings. The analysis indicates that this

diet also fails to correlate with increased sleep duration in the mutants. Additionally, the activity levels remain consistent with those observed in both control and mutant groups. This lack of significant activity change reinforces the notion that dietary protein levels do not impact sleep regulation in short sleeper flies.

3. **Medium Chain Fatty Acids**:

   - In **Figure C**, the introduction of medium chain fatty acids into the diet is examined. Similar to the previous diets, the data shows that the addition of fatty acids does not result in any sleep induction among the mutant flies. The activity levels remain comparable to those of the mutants on standard media, indicating that these fatty acids do not facilitate sleep in this particular model.

Summary of Findings

Overall, the consistent lack of sleep induction across all dietary interventions tested highlights a crucial characteristic of the short sleeper mutant flies. The absence of significant differences in activity levels between the controls and the mutants suggests that these flies maintain a state of restlessness regardless of dietary composition. This lack of response to various diets indicates that sleep regulation in short sleeper mutants may be largely independent of the nutritional factors tested, prompting further investigation into the underlying mechanisms that govern sleep in these genetically modified flies.

These findings can have broader implications for understanding sleep regulation and the potential influence of diet on sleep in other organisms, including humans. Future research could explore different dietary components or combinations that might interact with genetic factors to influence sleep behavior in Drosophila and beyond.

Objective 2 –

<u>Understanding association between activity and sleep in Obstructive Sleep Apnea (OSA)</u>

Obstructive Sleep Apnea (OSA) is a prevalent sleep disorder characterized by recurrent episodes of airway obstruction during sleep, leading to fragmented sleep and reduced oxygenation. This condition is often associated with excessive daytime sleepiness, cognitive impairment, and increased risk of cardiovascular diseases. Understanding the relationship between activity and sleep in individuals with OSA is crucial for developing effective management strategies.

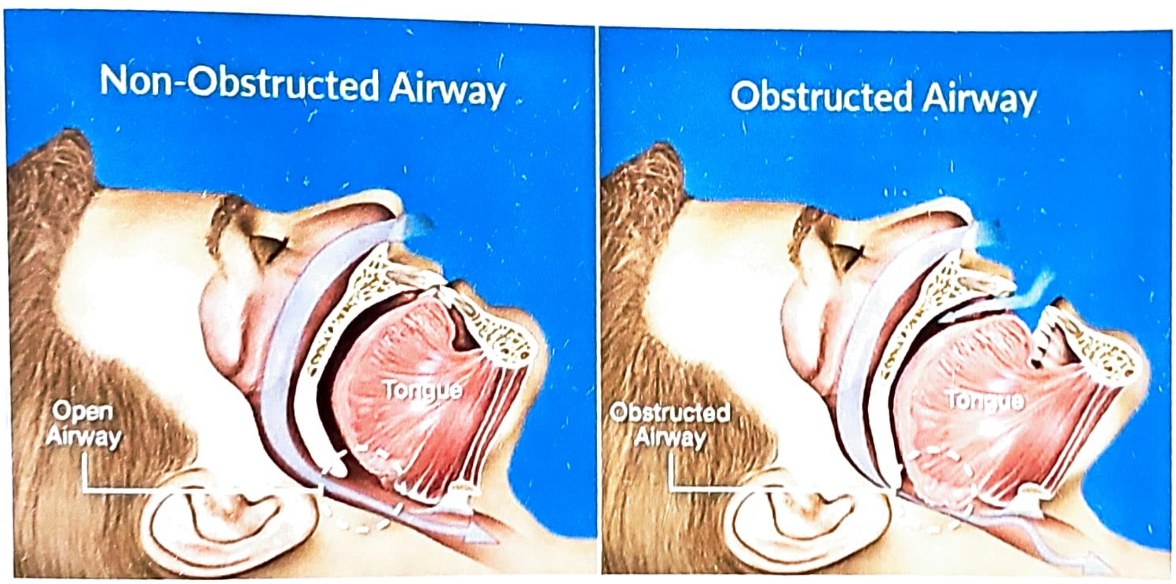


***Figure**: showing the reason for OSA.*
Source of image: Central Dental Avenue

Abstract 2 –

Obstructive Sleep Apnea (OSA) is a common sleep disorder marked by repeated airway obstruction during sleep, resulting in fragmented sleep and diminished oxygenation. This condition is closely linked to various health issues, including cardiovascular disease and cognitive impairment. Understanding the association between physical activity and sleep in individuals with OSA is essential for effective management and intervention strategies. Evidence suggests that regular physical activity can improve sleep quality, enhance respiratory function, and reduce the severity of apneic events. Moreover, exercise may facilitate weight management, a critical factor given the strong correlation between obesity and OSA severity. The timing and intensity of physical activity also play pivotal roles; moderate exercise earlier in the day can promote better sleep, while vigorous activity close to bedtime may disrupt sleep patterns. Recent advancements in wearable technology enable continuous monitoring of sleep and activity, providing insights for personalized treatment approaches. This underscores the importance of integrating physical activity into the management of OSA, highlighting its potential to improve sleep outcomes and overall health in affected individuals. Further research is warranted to explore optimal exercise regimens tailored to enhance sleep quality and mitigate the impacts of OSA.

Methods used for the experiment –

1. LC-MS (liquid chromatography mass spectrometry) –

Liquid Chromatography-Mass Spectrometry (LC-MS) is a powerful analytical technique widely used for the separation, identification, and quantification of complex mixtures. Combining the separation capabilities of liquid chromatography (LC) with the detection and structural elucidation features of mass spectrometry (MS), LC-MS has become essential in various fields, including pharmaceuticals, environmental science, food safety, and proteomics.

Components and Process

1. Liquid Chromatography:

- In LC, samples are injected into a chromatographic column packed with stationary phase material. As the mobile phase (a liquid solvent) flows through the column, components of the sample separate based on their interactions with the stationary phase. Different compounds elute at different times, known as retention times.

2. Mass Spectrometry:

- After separation, the eluted compounds are ionized and introduced into the mass spectrometer. In this step, ions are generated from the analytes, which are then sorted and detected based on their mass-to-charge ratio (m/z). This allows for the identification and quantification of the compounds.

Figure: *LC-MS machine.*

2. Actigraph data analysis –

Actigraph data analysis involves the interpretation of data collected from actigraphy devices, which are wearable sensors used to monitor physical activity and sleep patterns. These devices typically track movement through accelerometers, providing valuable insights into a person's daily activity levels, sleep duration, and quality.

Key Aspects of Actigraph Data Analysis

1. **Data Collection**:
    - Actigraphy devices are worn on the wrist or other body parts and record movement data over extended periods, often ranging from days to weeks. The data is usually sampled at regular intervals (e.g., every minute).
2. **Data Processing**:
    - Raw acceleration data is processed to remove noise and artifacts. This may involve filtering and segmenting the data to distinguish between different types of movement (e.g., sedentary, moderate, vigorous).
3. **Activity Metrics**:
    - Common metrics derived from actigraph data include:

- **Total Activity Counts**: Overall movement over a specified period.
- **Intensity Levels**: Classification of activity intensity (sedentary, light, moderate, vigorous).
- **Steps Count**: Total number of steps taken.
- **Time in Different Activity States**: Duration spent in various intensity levels.

4. **Statistical Analysis**:
   - Various statistical methods can be employed to analyze actigraph data, such as:
     - Descriptive statistics to summarize activity and sleep metrics.
     - Correlation analysis to examine relationships between activity levels and sleep quality.
     - Regression models to identify predictors of sleep patterns or activity levels.
5. **Applications**:
   - Actigraph data analysis is utilized in numerous fields, including:
     - **Clinical Research**: To study the effects of sleep disorders, mental health conditions, or chronic diseases.

- **Public Health**: To monitor population activity levels and promote healthier lifestyles.
- **Sports Science**: To optimize training regimens and recovery strategies for athletes.

Figure: *Image of binning software, i.e. ActiLife software being used.*
Source of image: actigraphcorp.my.site.com

Results 2 – OSA v/s Control subjects data –

Morning Activity and OSA Risk

1. **Circadian Rhythm Alignment**:
   - Individuals who are more active in the morning tend to have better-aligned circadian rhythms. Morning activity supports the body's natural biological clock, promoting a regular sleep-wake cycle. This alignment can lead to improved sleep quality and a lower risk of sleep disturbances, including OSA.
2. **Body Composition and Weight Management**:
   - Morning activity, especially when it includes cardiovascular exercise, can help regulate body weight. Maintaining a healthy weight is crucial because obesity is a significant risk factor for OSA. Excess weight, particularly around the neck, can increase the likelihood of airway obstruction during sleep.
3. **Muscle Tone and Respiratory Function**:
   - Engaging in physical activity in the morning can enhance muscle tone and respiratory function. Improved muscle tone in the upper airway can reduce the chances of airway collapse during sleep, thereby lowering the risk of OSA.

Afternoon Activity and Increased OSA Risk

1. **Delayed Sleep Onset**:
   - Individuals who are more active in the afternoon may experience delayed sleep onset, especially if their exercise routines are vigorous. Engaging in physical activity too close to bedtime can interfere with the ability to fall asleep and achieve restorative sleep, potentially exacerbating OSA symptoms.
2. **Weight and Fat Distribution**:
   - Afternoon activity patterns may not support effective weight management as effectively as morning activity. People who are less active in the morning may have a sedentary lifestyle, leading to weight gain, particularly in the upper body, which is a known contributor to OSA.
3. **Disrupted Sleep Quality**:
   - Those who are active later in the day may experience altered sleep architecture. For example, they might enter lighter sleep stages rather than deep sleep, where the body can effectively manage airway stability. This disruption can lead to increased episodes of apnea and hypopnea during the night.

Conclusion –

The timing of physical activity appears to have significant implications for the risk of Obstructive Sleep Apnea. Morning activity aligns well with circadian rhythms, supports weight management, and enhances muscle tone, thereby reducing the likelihood of OSA. In contrast, those who are less active in the morning and more active in the afternoon may experience disrupted sleep patterns and increased risk factors associated with OSA.

Promoting regular morning exercise and physical activity could be an effective strategy for reducing OSA risk, particularly for individuals predisposed to the condition. Further research is warranted to better understand the nuances of these relationships and to develop targeted interventions that incorporate activity timing into OSA management.

Bibliography-

Apart from a few images, all of the work is original, and is subject to confidentiality

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