

Introduction

1. Introduction

Circadian rhythm, also known as a circadian cycle, is a regular oscillation that happens every 24 hours on average (Reddy, S. et al., 2018). Circadian rhythms can refer to any mechanism that is endogenous, or originating from inside an organism, and that reacts to, or is influenced, by the environment (Fagiani, F. et al., 2022). A circadian clock, whose main purpose is to regularly coordinate biological activities so that they take place at the ideal moment to maximise an individual's fitness, controls circadian rhythms. Circadian rhythms have been widely observed in animals, plants, fungi, and cyanobacteria, and there is evidence that these rhythms separately originated in each of these kingdoms of life (Edgar et al., 2012).

Many physiological processes are timed to occur every 24 hours to prepare for the environment's shift from day to night and vice versa. According to (Meléndez-Fernández et al., 2023), these 24-hour regulations, also known as circadian rhythms, regulate the sleep-wake cycle, locomotor activity, and preparation for food availability during the active phase (during the day for nocturnal animals and the night for nocturnal humans). Some people may develop circadian rhythm disturbances as a result of environmental influences or sleep issues. Social jetlag or shift work that disrupts circadian rhythms at the organ or systemic level raises the risk of developing chronic metabolic illnesses like diabetes mellitus and neurological diseases (Peng et al., 2022).

1.1 Sleep wake cycle

Sleep-wake pattern is the best example of circadian rhythm over the course of a 24 hour of the day. It helps control daily schedule for sleep and wakefulness that most living things have one. Circadian rhythm is influenced by light and dark cycle, as well as other factors. Our brain receives signals from light and activates certain hormones, alters body temperature, and regulates our metabolism to keep us alert. We sleep during the night and wake up during the day time because we adapted to this environment since we were born (Okechukwu, C. E., 2022). On the other hand, new born babies do not have this sleep-wake pattern initially because they came from the constant dark environment of uterus and they slowly adapt to this sleep-wake pattern approximately after one year because the primary rhythmic regulator of this sleep-wake cycle is sunlight. The suprachiasmatic nucleus (SCN), receives signals from the optic nerve in our eyes, it then causes the production of cortisol, and other wake-up-inducing chemicals. However, when nighttime darkness arrives, the SCN sends a signal to the pineal gland, which then causes the production of a chemical called melatonin, which promotes sleep (Blume et al., 2019).

1.2 Common sleep deprivation consequences

The sleep wake cycle is a very important to maintain health because release of several hormones follows the rhythmic pattern based on day night cycle for example corticosterone (CORT), melatonin, leptin, and ghrelin. These hormones regulate the body physiology and metabolism. If we don't sleep during night, the release pattern of these hormones will disturb and leads to development of several metabolic and neurologic disorders (Peng et al., 2022) as well as several other problems like memory impairments, deterioration in decision-making ability and eye-hand coordination.

1.3 Difference between circadian rhythm and biological clock

Circadian rhythms and other body functions are timed in part by a biological clock. It is the body's intrinsic clock and is unaffected by outside influences. A biological clock's impact of a circadian rhythm is not a property shared by all biological clocks.

1.4 Different types of cycles

There are several types of circadian cycles as follows

1.4.1 Circadian rhythm

The Circadian rhythm is a 24-hour cycle; it is a part of the body's internal clock. They are running to carry our essential functions and processes of the body. One of the well-known circadian rhythms is the sleep-wake cycle. The different systems of the body follow the master clock of the brain, which synchronizes with circadian rhythms. The circadian clock plays an important role in a physical, mental, and behavioral role that depends on light and dark cycle (Reddy, S. et al., 2018).

1.4.2 Diurnal Rhythms

The diurnal rhythms are also known as endogenous rhythms. It syncs the day and night with circadian rhythms. It is mainly influenced by environmental clues like climate changes (Seney, M. L. et al., 2019)

1.4.3 Ultradian rhythm

The ultradian rhythms occur more than once a day. It lasts for short period at high frequency, For example, deep sleep for ninety minutes. The alertness and hormone level follow the ultradian rhythms (Gerkema, M. P., 2002).

1.4.4 Infradian/Circalunar rhythm

A biological rhythm, which lasts for more than 24 hours, is because of infradian rhythms. For example, the menstrual cycle in women occurs every 28 days (Pronina, T. S., 1992).

1.4.5 Circannual rhythm

Circannual rhythms are self-sustained endogenously driven biological oscillations that persist with a regular periodicity of about 12 months in the absence of environmental cycles (Miyazaki, Y. et al., 2015)

1.5 Circadian clock of photic and nonphotic cues

There are two passage ways leading to stimulate suprachiasmatic nuclei (SCN). The retinohypothalamic tract (RHT) and the geniculohypothalamic tract (GHT), which connects the retina and the geniculate cortex, are the photic input channels that transmit information about the intensity and spectral content of ambient light. The SCN also receives non-photoc signals from the pineal gland and raphe nuclei via the geniculohypothalamic tract (GHT), through the thalamic intergeniculate leaflet (IGL). The major output comes from the SCN to these raphenuclei, which receive information about the phase of the circadian clock and control the body's alertness state, and the pineal gland, which produces melatonin. Loops formed by input and output channels are reciprocal (Challet, E., and Pévet, P., 2003).

1.6 Type of disrupted circadian rhythm disorder

The timing of when a person sleeps and is awake is a concern in both circadian rhythm and sleep disorders. The brain's SCN control centre houses the

body's primary circadian clock. This internal clock controls how quickly physiological rhythms like temperature and hormone levels occur. The sleep-wake cycle is the main circadian rhythm that this body clock regulates. The cycle that the circadian clock runs on is just a little bit longer than 24 hours. The main visual signals of light and darkness that set the circadian clock are sent from the eyes to the SCN. This maintains the clock's alignment with the 24-hour day. The timing of the clock can also be influenced by additional time cues, or zeitgebers (ZTs). The timing of meals and workouts is one of these indications. As a person matures, their circadian rhythms and their sensitivity to time signals may alter.

Each circadian rhythm sleep disorder involves one of these two problems;

- have a hard time initiating sleep.
- Struggle to maintain sleep, waking up frequently during the night.
- Tend to wake up too early and are unable to go back to sleep.
- Sleep is non-restorative or of poor quality.

1.6.1 Delayed sleep phase disorder (DSP)

When a person routinely falls asleep and awakens more than two hours later than is deemed usual, this condition is known as DSP. The evening types who remain up until 1 or later in the morning and get up in the late morning or early afternoon are often those with DSP. A person with DSP will have a fairly stable sleep pattern if they can consistently go to bed at the recommended late hour. DSP has a reported incidence range of 7–16% and is more prevalent in teens and young adults. In sleep clinics, 10% or more of people with persistent insomnia are thought to have DSP. Approximately 40% of people with DSP may have a favorable family history.

1.6.2 Advanced sleep phase disorder (ASP)

ASP happens when a person consistently goes to bed and rises earlier than the majority of others. The "morning types" who have ASP often wake up between 2 and 5 in the morning and go to bed between 6 and 9 in the evening. A person with ASP will have a relatively stable sleep pattern if they can consistently go to bed at the chosen early time. In middle-aged and older individuals, ASP affects around 1% of them, and its prevalence rises with age.

1.6.3 Jet lag disorder

Jet lag happens when a person abruptly enters a different time zone after a lengthy flight. The individual must sleep and wake up in this unfamiliar place at times that doesn't correspond to their biological clock. The more time zones that are traversed, the more serious the issue becomes. The body often adjusts to eastward travel more readily than westward travel. All ages are affected by jet lag. However, compared to younger individuals, older people may experience more severe symptoms and a slower pace of recovery. Transmeridian travel-related insomnia can become more severe and lead to decreased alertness and function. Other factors that might contribute to this include sleep loss, protracted uncomfortable seating positions, air quality and pressure, stress, and excessive coffee and alcohol usage. The symptoms of jet lag start around one to two days following flight travel that involves crossing at least two time zones. By reversing the circadian rhythms, improper light exposure can extend the time it takes for the body to reset.

1.6.4 Shift work disorder

Shift work disorder occurs when a person's work hours are scheduled during the normal sleep period. Sleepiness during the work shift is common, and trying to sleep during the time of day when most others are awake can be a struggle. Shift-work

schedules include night shifts, early-morning shifts and rotating shifts. Depending on the type of shift, diurnal or circadian preferences may influence the ability to adjust to shift work. For example, individuals described as morning types appear to obtain shorter daytime sleep after a night shift. Persons with co-morbid medical, psychiatric and other sleep disorders such as sleep apnea and individuals with a strong need for stable hours of sleep may be at particular risk.

1.6.5 Irregular sleep-wake rhythm disorder

When a person's sleep-wake cycle is irregular, this condition manifests. Over the course of a day, the person's sleep is broken up into a number of naps. People with the condition complain of excessive or persistent tiredness. Children with mental retardation and neurological illnesses like dementia may also be associated with low-amplitude or irregular circadian rhythms of sleep-wake patterns.

1.6.6 Free-running (nonentrained)

When a person's varied sleep-wake cycle changes later each day, this condition manifests. When the brain receives no illumination cues from the surroundings, it frequently occurs. The condition can occasionally coexist with dementia or mental impairment. Additionally, it has been proposed that the delayed sleep phase type of circadian rhythm sleep disorder and the free-running form of the disorder may overlap.

1.7 Transcription/Translation Feedback Loops (TTFL) of the Mammalian Clock

Clock genes are controlled by the proteins that are produced from them in a negative feedback loop known as the transcription/translation feedback loop (TTFL).

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Positive regulatory elements that stimulate transcription and protein products that repress transcription typically make up the two major arms of the TTFL. An mRNA transcript is produced as a consequence of transcription when a positive regulatory element binds to the clock gene promoter, and a protein is produced as a result of translation. Due to translation dynamics, post-translational protein modification, protein dimerization, and intracellular movement to the nucleus, there is a distinctive delay between the accumulation of mRNA transcripts and the accumulation of proteins, as well as gene suppression (Hastings et al., 2008). Once the cytoplasm has accumulated enough changed protein products, they are carried into the nucleus where they block the positive element from the promoter to halt clock gene production. Thus, the clock gene is transcribed at low levels until the degradation of its protein products, when positive regulatory elements attach to the promoter and transcription may be restarted. Numerous characteristics of the TTFL's negative feedback loop are crucial for the cellular circadian clock. The delay between translation and the gene's negative regulation causes daily cycles in both gene transcription and protein quantity and size. Each individual's cycle period, or the amount of time needed to finish one cycle, is constant and barring mutation, is usually close to 24 hours. This makes it possible for sustained entrainment to the earth's natural 24-hour cycle of light and darkness. Additionally, clock genes can establish daily rhythms in other biological processes, including metabolism, inside the organism since their protein byproducts regulate downstream genes that are not a part of the feedback loop (Hastings et al., 2008). The TTFL is also a limit cycle, which means that it is a closed loop that will always resume following its predetermined course, maintaining the oscillatory path during its predetermined 24-hour period (Sheredos, 2013).

1.8 Importance of light as a source of energy

It has been demonstrated unequivocally that red lights boost the efficiency and improve the energy and ATP synthesis of the mitochondria (Ferraresi et al., 2015). This is only one instance of how biological systems, notably the mitochondria, use sunlight to significantly increase energy production. And so, when people say, let's do some red-light therapy and improve our mitochondrial function, it is obvious that in nature, we would have this advantage from the sun continually optimizing our mitochondrial function because we have evolved with it. But as a result of our shift to an indoor, technology-based lifestyle where we are seriously lacking in red and near-infrared light, we no longer actually have it.

1.9 Circadian control of mitochondrial respiration

All organs have different energy needs depending on how active they are. Additionally, the use of substrates might vary between active and passive phases of fat, protein and carbohydrate oxidation. As a result, preparing the mitochondrial respiratory capacity for the light and dark phases would be effective. Since peripheral oscillators employ unique entrainment cues, such as food and exercise, clock-controlled gene expression shows significant organ specificity. The effect of pervasive environmental variables, most notably nutrition availability, which likewise exhibit daily rhythmicity, is a significant confound in the study of clock-controlled mitochondrial functions. In addition to the biological clock, additional variables including eating and activity states as well as the light-dark cycle regulate the majority of in vivo operations. Separating the effect of the biological clock from all other factors on behavior is important in order to examine the biological clock's

distinct contribution. Numerous methods have been accessible during the past several decades, making it possible to investigate the biological clock at the system and organ level. The biological clock can be genetically disrupted in mouse models to explore the isolated effects of a disrupted clock on mitochondrial functions.

1.10 Light source that we exposed in our daily life and its consequences

People have been accustomed to being exposed to both high intensity sun light (about 100,000 lux) during the day and low-intensity moonlight (0.1-0.3 lux) during the full moon phase on a clear night for a very long time (Takemura et al., 2004). Contrarily, throughout the past century, exposure to artificial light has spread throughout the industrialised globe, with around 99% of the people in the US and Europe reporting substantial night-time lighting (Cinzano et al., 2001); (Bedrosian et al., 2011); (Navara & Nelson, 2007). Due to lighting in homes and businesses, individuals now encounter lighting with a significantly lower intensity (400-600 lux) during the day and a greater illumination (100-300 lux) at night (Elliott et al., 2006). Additionally, at night, people utilize light-emitting gadgets (such as tablets, cell phones, and laptops) that release between 30 and 50 lux of light (Chinoy et al., 2018). Furthermore, nocturnal light exposure is becoming more prevalent in contemporary life, and there are several factors contributing to this, including shift employment and watching television. In addition, many individuals keep a light on while they sleep to make it easier to care for young children or elderly family members (Bedrosian & Nelson, 2017). Further, the majority of international corporations now operate day and night, and employees spend the entire night in the light while working. Younger people have a strong propensity for working late hours, both in India (Wai & Langer, 2016) (Verma, 2011) and abroad (Rydz et al., 2020); (Patel, 2006). This exposes them

to low levels of artificial light at night. So that later on they suffer from many diseases i.g., metabolic disorders: diabetes mellitus, (Cornelissen et al., 2018); (Yoshino & Klein, 2013), obesity (Brum et al., 2020), cardiovascular diseases (Dominguez-Rodriguez et al., 2009), and cancer (Savvidis & Koutsilieris, 2012). In addition to disrupting circadian rhythmicity, long trips that cause jet lag, nighttime work, and exposure to artificial light during the night can also cause irritation, anxiety, and depressed behavior (Salgado-Delgado et al., 2011). In this context, animal models of light-dark cycle-controlled alterations have advanced our knowledge of the relationships between circadian rhythmicity and physiological systems including metabolism and cognition. People are exposing to the night time light in their daily life routine. Thus, the biological rhythms may not be well-tuned to the environmental cycles, with potential negative consequences on behaviour, physiology and performance. Changes in environmental cycles of light/dark and timing of food intake, induces internal desynchronization of circadian clocks so that physiological and behavioral rhythms are no longer optimally tuned to environmental conditions (Yamazaki et al., 2000).

1.11 Mitochondrial modulators

1.11.1 N-Acetylcysteine (NAC)

N-acetylcysteine (NAC) is a derivative of a thiol-containing amino acid, which directly or indirectly increases the concentration of glutathione and demonstrated to have an antioxidant property. NAC is a cysteine donor for the synthesis of glutathione (GSH). It comes from the amino acid L-cysteine that is a part of the building blocks of proteins. It is a glutamate modulator that is devoid of the adverse effects of all the common to most NMDA antagonists, (Costa-Campos et al.,

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2013); (Ferreira et al., 2010). NAC has many uses and is a FDA approved drug. It is used by healthcare providers to treat acetaminophen (paracetamol) poisoning. It works by binding the poisonous forms of acetaminophen that are formed in the liver. It is a strong antioxidant and effective in several antioxidants therapy.

1.11.2 Melatonin

Melatonin is a hormone made in the body. It regulates night and day cycles or sleep-wake cycles. Melatonin in supplements is usually made in a lab. Darkness triggers the body to make more melatonin, which signals the body to sleep. Light decreases melatonin production and signals the body to be awake. Some people who have trouble sleeping have low levels of melatonin. It's thought that adding melatonin from supplements might help them to sleep. People most commonly use melatonin for insomnia and improving sleep in different conditions, such as jet lag. It is also used for depression, chronic pain, dementia, and many other conditions.

1.12 Rationale

- There is lack of understanding of effect of disruption in circadian rhythm on mitochondrial bioenergetics in the SCN
- There is no pharmacological intervention for correcting mitochondrial bioenergetics during disruption of the circadian rhythm in central and peripheral clock
- Pharmacological investigation of effect of N-acetylcysteine (NAC) in circadian rhythm disruption induced mitochondrial dysfunction

1.13 Lacunae in the existing literature and relevance of the study

The literature shows scattered and incomplete information on the role of mitochondrial bioenergetics on circadian rhythm and other biological system and the information is inconclusive. There was no study reported so far that explains the underlying causes of the involvement of mitochondria in the circadian rhythm and related disorders. As the sleep cycle is an important phenomenon for human behavior. There are no preclinical evidences on chronic dim light exposure on mitochondrial function and circadian rhythm in SCN. Further, there is limited research on the mechanism of NAC and melatonin on mitochondrial respiration in circadian rhythm. In other associated diseases there are well known literature are provided evidence that mitochondrial dysfunction is the associated with the several metabolic diseases like obesity (Bhatti et al., 2017) and neurological diseases like Parkinson, (Park et al., 2018), Alzheimer (Ashleigh et al., 2023) and Huntington disease (Browne., 2008). Therefore, a ‘Study of light induced circadian disruption and mitochondrial dysfunction, the possible causes involved in the pathophysiology changes in SCN and other peripheral organ such as liver, kidney, heart, and muscle mitochondria following dim light exposure during night are needed. Based on the above information the hypothesis has been postulated.

1.14. Hypotheses

The study focuses on the effect of N-acetylcysteine (NAC) against chronic dim light induced circadian rhythm disruption and mitochondrial dysfunction in both central as well as peripheral clock, as well as the role of mitochondria in normal and disrupted circadian rhythm. Chronic dim light act as a stressor by increasing the

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CORT and decreases the melatonin level, this may lead to increase free radical load in the brain causing oxidative stress. Under the oxidative stress condition cells will be deprived of energy which is an indication of mitochondrial dysfunction. NAC reported/used in clinical studies as a potent antioxidant which can neutralize the free radical and improves energy within the tissue and organ (Ezeriņa et al., 2018).

On the other hand, chronic dim light exposure may decrease the level of GSH in the brain as well as in the liver, Hence, GSH is a physiological reservoir of glutamate (an excitatory neurotransmitter), (Koga et al., 2011). So the level of glutamate increases due to insufficiency of GSH in the brain of chronic dim light exposed mice. Release of glutamate up-regulates both N-methyl-D-aspartate (NMDA) and amino-methyl propionic acid/kainate (AMPA/KA), the ionotropic glutamate receptors. It is well established that NMDA/AMPA/KA plays a critical role in mediating the effects of light on the circadian system through CLOCK/BMAL1 and CRY1/CRY2 gene (Michel et al., 2002). Hence, NAC is a precursor for the synthesis of GSH; it donates the thiol group to fasten the GSH synthesis which is a natural antioxidant inside the body and maintains the glutamate level in the brain. In the peripheral clock the most important organ is liver because it is responsible for the metabolism of food material to generate energy. Chronic dim light exposure reduces GSH and mitochondrial enzyme succinate dehydrogenase (SDH), along with the hunger hormone leptin and ghrelin which regulates the metabolism of food and this leads to disturbance of the circadian rhythm and the feeding/fasting mechanism (Pickel and Sung, 2020).

Hence, NAC is reported to have a potent antioxidant property by neutralizing free radicals, and improvement in GSH synthesis. Thus, we hypothesized that NAC may be a potent target to correct the disrupted circadian rhythm by improving the

mitochondrial GSH and SDH enzyme in the central as well as in peripheral clock. Further there several reports are showing that mitochondria are involved in the circadian rhythm in the tissue clock like heart, liver and muscles and some *in-vitro* studies, but there is no evidence that such mitochondrial function is following the circadian rhythm pattern in the master clock (SCN). So we sought to evaluate the mitochondrial bioenergetics and mitochondrial DNA for its function in normal and disrupted circadian rhythm. Also there are not much known mitochondrial modulators are available. Thus, there are many drugs that's have the ability to improve mitochondrial function but they need a proper screening method to evaluate their exact mitochondrial effect. Hence, we sought to develop an easy, robust and economical *ex-vivo* method for screening the drugs those promising to have some mitochondrial modulating property.

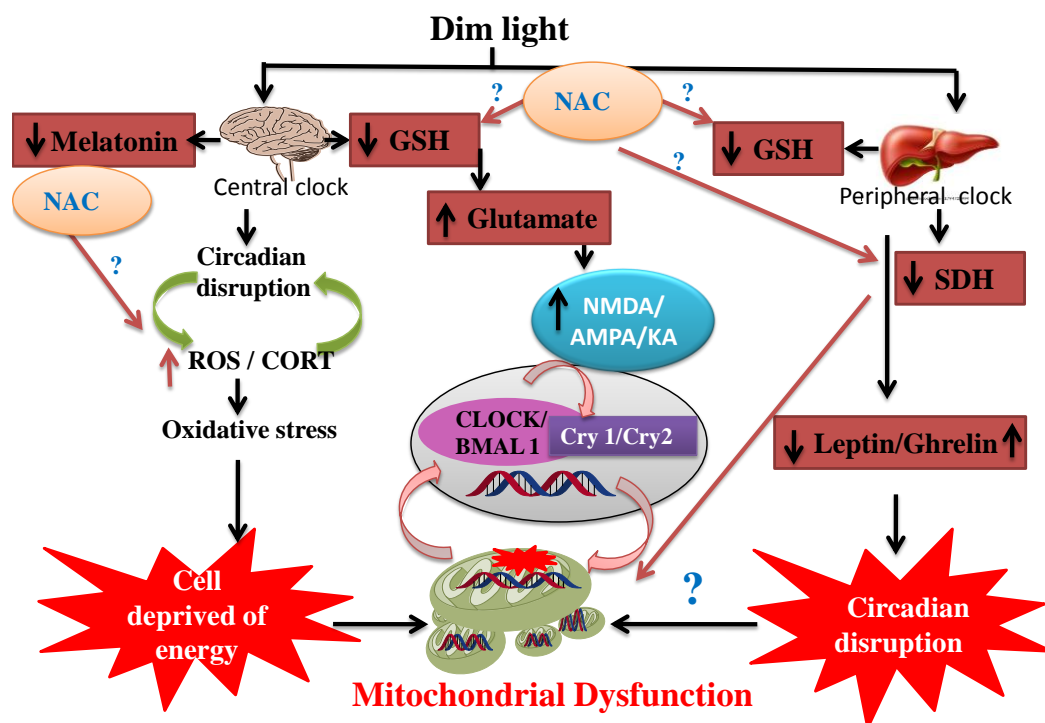


Figure 1.1 Proposed hypothesis

1.15 Objectives

1. Evaluation of the role of mitochondria in normal and disrupted circadian rhythm.
2. Evaluation of effect of N-acetylcysteine on central clock related circadian rhythm.
3. Evaluation of effect of N-acetylcysteine on peripheral clock related circadian rhythm.
4. Development of an *ex-vivo* method for evaluation of mitochondrial modulators.