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SAMAY

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JAN 2024

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INSIDE...

Editor's Note

Dear Readers,

The editorial team of the biannual newsletter of the Indian Society for Chronobiology (InSC) wishes you all a very Happy New Year 2024. We hope that this year brings happiness and new opportunities for all of us and our future endeavors culminate into favorable outcomes.

Along with the member of the editorial team of SAMAY, I am happy to bring you the January 2024 issue of our bi-annual newsletter. We are starting this issue by remembering and honoring the lives and contributions of two of our dear members: Prof. P. Subramanian and Dr. K. K. Sharma. This issue is focused on “Recent advances in fly clock research”, under which we have included various interesting articles, staring with the research journey of Prof. Sheeba Vasu who has recently been awarded a fellowship of the Indian National Science Academy (INSA). The InSC community feels proud of her and wishes the best for her future endeavors. Other articles discuss how *Drosophila* is a great model system to study circadian rhythms and how it is being utilized. Finally, we have included a section on "Upcoming events" to keep you up-to-date.

The credit for successful publication of this newsletter goes to the editorial board for putting together this issue and to all the members and researchers who contributed and helped us fulfill the deadline. We extend our thanks and appreciation to all the contributors for their prompt replies to our requests.

Please drop us a line (at inscdu@gmail.com) with your suggestions on topics you'd like to see us cover or things that you like or do not like about what we are doing. We look forward for your feedback and suggestions.

Warm Regards
Sangeeta Rani
Editor-in-chief, SAMAY
Indian Society for Chronobiology

Remembering Professor P. Subramanian

- G. Marimuthu, INSA Honorary Scientist
Madurai Kamaraj University, Madurai 625 021 (Email: emailboxgm@gmail.com)

It is difficult to believe the fact that our beloved member of the Indian Society for Chronobiology (InSC), Professor P. Subramanian is no longer with us. He sadly died on 19th September 2023, due to a sudden heart attack.

Prof. Subramanian was an active member of the InSC, served as member of the Executive committee several times, and also as the Vice-President of the InSC during the period 2006-2008. He always contributed to the growth of the subject in the country, and organized 4th SERC School in Chronobiology in the year 2005.

Prof. Subramanian was born on 27th July 1964 in Madurai. He obtained BSc (Chemistry and Botany – double major) from the Madura College, Madurai during the year 1984. Then he joined the School of Biological Sciences (SBS), Madurai Kamaraj University to study MSc (Integrated Biology) and got the degree in 1986. Afterward, he joined the Department of Animal Behavior and Physiology, SBS to carry out research under the supervision of late Prof. R. Subbaraj (former President of the InSC). His Ph.D. work was on “Studies on circadian locomotor activity rhythm in the field mouse, *Mus booduga*: effect of psychoactive drugs”. He received the Ph.D. degree in 1993. Afterwards he joined the Department of Biochemistry and Biotechnology, Annamalai University as a Lecturer. After several years of service, his position was elevated to Professor and Head of the Department. He passed away by leaving behind one more year of service before the formal retirement.

Prof. Subramanian’s research interest was on molecular chronobiology, hepatotoxicity, nephrotoxicity and cancer biology. He supervised around 22 Ph.D. students during his tenure. A couple of students still wait for their thesis defense. He had completed eight research projects that were supported by DST, UGC, ICMR and DRDE. He also handled a collaborative project with University of Malaya. He had published 120 papers in peer-reviewed journals and six book chapters. He had organized several conferences, including the XVI National Symposium on Chronobiology (October 2004). He had participated almost in all conferences of the InSC. He served as a Resource Person in Refresher Courses that were organized in several universities.

As a human being, Prof. Subramanian was a calm, polite and humble person. Very sincere in his work – both in teaching and research. His departure is a great loss to the Indian Society for Chronobiology. He will ever be remembered by the biochemists and chronobiologists.

Remembering Dr. K. K. Sharma

- Prof. Dinesh Bhatt, Professor Emeritus,
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Dr. K.K. Sharma was born on 06 June, 1953 in Bhagalpur, Bihar. He lived his life in a very humble manner and passed away peacefully at his home on 06 December, 2023.

Dr. Sharma did his post-graduation in Zoology from Patna University. Thereafter he joined Jamshedpur Co-operative College, Jamshedpur, Jharkhand, as a faculty in the Department of Zoology in 1976.

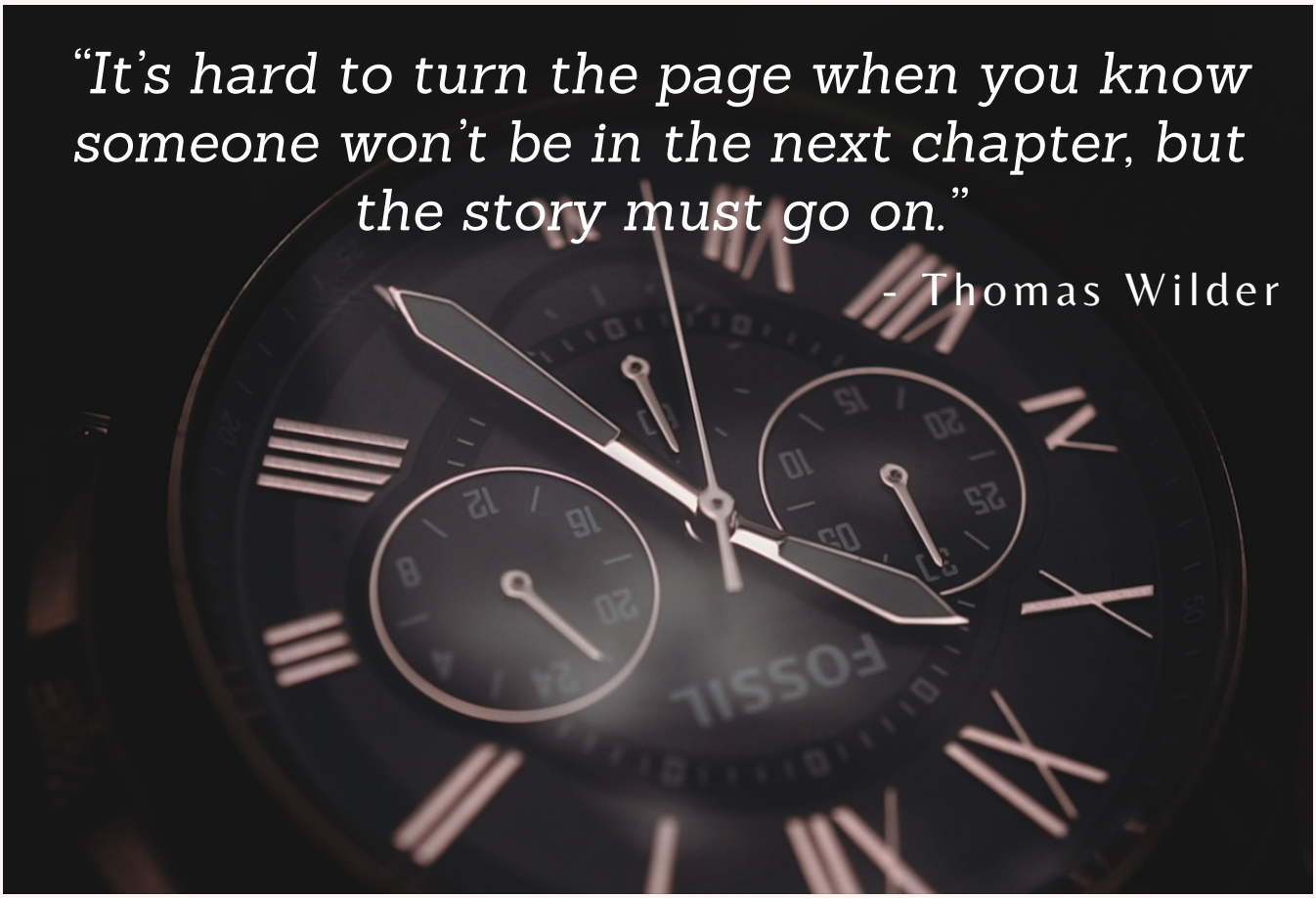
He had an admirable research carrer. He was awarded JRF and Teacher-Fellowship by CSIR and UGC in 1977 and 1981, respectively. Under the UGC Scheme of Teacher Fellowship, he joined the lab of Dr. Asha Chandola, Department of Zoology, BHU, Varanasi as a Ph.D. scholar and completed his work in 1985. While at BHU, he worked with then the lab colleagues namely P. P. Pathak, Sushama Pavgi, Anand Kar, Krishna Chakravorty, and myself. I have known him since then.

His research interests centered around field biology, particularly in avian ecology, sociobiology, biodiversity conservation and environmental education. Despite limited facilities in the college, he successfully supervised four PhDs. Also, he acted as the Principal Investigator in major research projects funded by DST, New Delhi. He was a life member of the Indian Society of Chronobiology and Association of Avian Biologists in India. Dr. Sharma published his findings in several reputed journals. Besides, he contributed various popular science articles in prestigious newspapers such as The Telegraph, Hindustan Times, and Economic Times.

Dr. Sharma was a brilliant student and hence, he was awarded Govt. fellowships throughout his academic career. He was a popular teacher among the students because of his deep knowledge of the subject and excellent communication skills. He was greatly influenced by the art of music and used to sing Ghazals and religious songs. He was indeed very kind, polite and distinguished fellow

and had an inherent quality to live for the people and friends around him.

During my Ph.D. work whenever I encountered with any problem, K.K. was the first fellow available with the solutions along with his sense of humour, wisdom and experience. I recall the time when in 1982, our mentor Prof. Asha Chandola joined the Garhwal University, Srinagar as a faculty and K.K. and I also had to shift to Garhwal University for thesis – writing/checking. One evening, I visited the lab after having returned from the field, K.K. delivered me an interview letter for the post of lecturer at Gurukula Kangri University, Haridwar for which I had applied a few months back. It was the last days of the month and I was not able to afford the travel expenses. I declined to appear for the interview due to scarcity of money and my interest in joining a lab abroad as a PDF rather than the Gurukula University. He stimulated and insisted me to appear for the interview with a wise suggestion - 'You can go abroad anytime in your life but to get a permanent position in India is not easy'. I was convinced with his words and attended the interview with the travel assistance offered by K.K. How can I forget his wise suggestion and kind gesture. He will be remembered and missed by us till we live. Unfortunately, I did not get an opportunity to interact with his family.



INTERNATIONAL RECOGNITIONS TO OUR ESTEEMED EC MEMBER: PROF. MEWA SINGH

Congratulations

The Indian Society for Chronobiology feels immense pleasure in sharing the latest international recognition of one of its executive members, Prof. Mewa Singh, who is a Distinguished Professor (for Life), SERB Distinguished Fellow and INSA Distinguished professor (designate) at the Institution of Excellence, Vijnana Bhavan, University of Mysore, Manasagangotry, Mysore.

Professor Singh has been conferred the 2023 ATBC Honorary Fellowship by the Association of Tropical Biology and Conservation, and awarded Lifetime Achievement Award by the International Primatological Society. This adds to a long list of fellowships from the Indian Academy of Sciences Bangalore, The National Academy of Sciences Allahabad, Indian National Science Academy Delhi, and the National Academy of Psychology India.

These recognitions have been bestowed upon him for his ability to inspire and support many researchers in India and abroad, and for his significant contributions in mentoring and inspiring the next generation of researchers. His research has focused on behavior and ecology of several mammal species, with a particular focus on primate social behavior, apart from interdisciplinary topics such as urban ecology, biodiversity conservation, and human-wildlife interactions. As truly indicated in his ATBC nomination letter “It would not be an exaggeration to state that it would be impossible to work on a mammal species in India without citing or referring to at least a few papers by Prof. Mewa Singh or his students.”

The InSC family congratulates Prof. Singh for these honors from the International Science Community, and wishes him well for his future endeavors.

FOCUS: RECENT ADVANCES IN FLY CLOCK RESEARCH

Drosophila melanogaster is a great model system to study circadian rhythms. At different stages of its life, multiple output rhythms of the clock can be measured and characterized with relative ease

-Surajit Dawn
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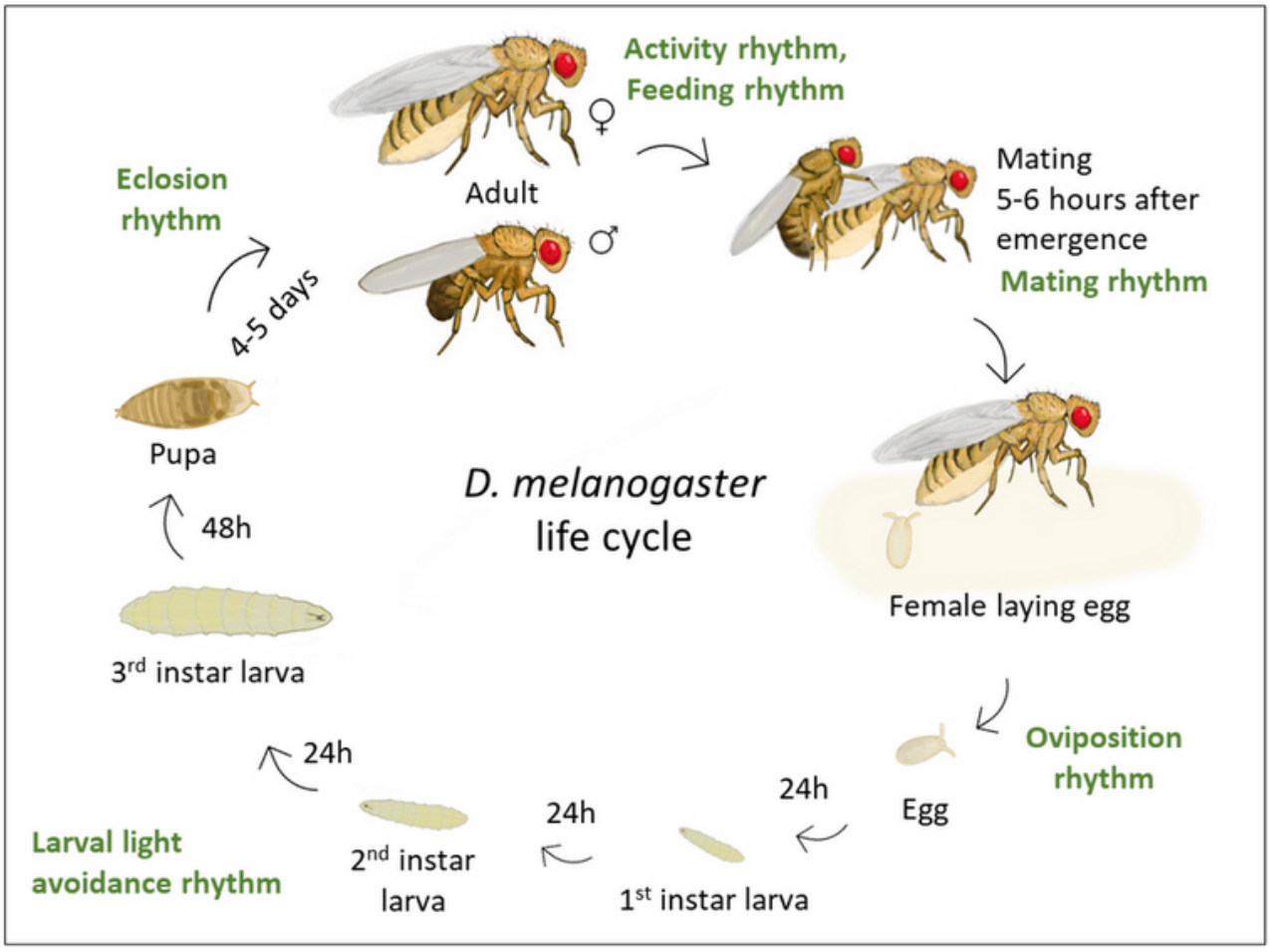

Fly models to study circadian clock evolution and neuronal circuitry

Sheeba Vasu
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I began my research career as an independent researcher at the Jawaharlal Nehru Centre for Advanced Scientific Research in January 2009 and have been fortunate to receive support from members of the chronobiology community in India and abroad in receiving suggestions, reagents and collaborations. Over the years, I have been lucky to have a bunch of enthusiastic and hardworking members join my research group. Together we have been studying daily rhythms and their underlying biological clocks from both an evolutionary as well as neurobiological perspective. I summarize here a few studies from our group, which have contributed to the body of knowledge on circadian rhythms. These were possibly the basis of my being considered for the fellowship of the Indian National Science Academy (INSA) recently.

Priya Prabhakaran, my first PhD student used a comparative approach to examine locally caught populations of Drosophilids for various rhythmic behaviours including their locomotion and eclosion. Using various behavioural assays, we were able to show that closely related sympatric species can occupy distinct temporal niches and have distinct circadian clock properties as reflected by studies under laboratory light-dark or warm-cold cycles (Prabhakaran & Sheeba 2012, J Biol Rhythms; 2013, J Exp Biol, 2014a, J Comp Physiol A: 2014b, Chronobiol Intl).

Sleep is a biological process whose mecha-nistic and functional underpinnings are poorly understood. Sleep is known to be under dual control - the circadian clock, and homeostatic processes which together dictate the timing, duration and quality of sleep. Flies have proven to be a very valuable tool to study genetic and neuronal circuitry underlying both circadian rhythms and sleep and studies by PhD student, Sheetal Potdar, showed evidence for a circadian neuropeptide receptor PDFR to communicate wakefulness to sleep circuits via dopaminergic neurons (Potdar and Sheeba 2018, eNeuro). While it is evident that sleep disruption has detrimental consequences, how ill health is caused, is not clear. Using a multipronged approach – pharmacological, mechanical, and genetic tools to cause sleep deprivation, Sheetal along with enthusiastic summer interns showed that loss of sleep greatly affects egg laying by female flies demonstrating that sleep loss can have consequences to reproductive fitness in animals (Potdar et al., 2018, J Exp Biol). Recently a collaborative study with Dr. Shahnaz Lone (GITAM University) helped us to reveal a role for mechanosensory structures and their neuronal circuity contributing to sleep induction due to gentle orbital motion in flies (Lone et al., 2021, J Neuroscience).

Multiple studies from our group have

aimed to dissect the neuronal circuit that regulates rhythmic locomotion in flies. Studies by Antara Das have demonstrated the role of a temperature sensitive ion channel dTRPA1 in enabling flies to appropriately phase their activity in the presence of cyclic temperature or constant warm ambient temperatures and cycling light levels. They have shown that this circuit can generate contrasting behavioural responses – inhibition versus enhanced locomotion in the face of warm temperatures dependent on the pattern of temperature cycles – abrupt versus gradually changing temperatures (Das et al., 2015 PLoS One; 2016, J Biol Rhythms). Taking this further, Aishwariya Iyengar showed that a subset of circadian pacemaker neurons is critical for the suppression of nighttime activity when ambient temperatures remain warm throughout the day (Iyengar et al., 2022, Genes Brain Behavior).

Circadian clocks are known to be strong regulators of metabolic processes and in collaboration with Kavita Dorai (IISER, Mohali), an NMR-based approach revealed many new components of the *Drosophila* metabolome that cycle when only light and temperature are provided as time cues (Gogna et al., 2015, Molecular Biosystems). More recently, Viveka Singh showed that in contrast to many vertebrate models, food availability cycles do not act as an entraining agent 'zeitgeber' to the central clock in flies (Singh et al., 2022, J Biol Rhythms).

While the fly circadian pacemaker circuit has been the focus of research by multiple groups around the world for several decades, thus far, chemical synapses are the only type of communication reported. Recent studies by Aishwarya Ramakrishnan suggested that gap junction forming proteins - Innexins expressed in the fly brain influence the speed of the cellular molecular clock and behavioural locomotor activity rhythms (Ramakrishnan and Sheeba, 2021 iScience).

Pavitra Prakash used the fly circadian pacemaker neurons to examine the cellular processes that underlie progression of neurodegenerative conditions which are often caused by misfolding of certain proteins which eventually cause defective proteostasis. She showed that in this fly model, harnessing heat shock proteins can ameliorate the toxicity of a mutated version of the human Huntingtin gene which is implicated in the debilitating human genetic disorder Huntington's Disease (Prakash et al., 2022, Disease Models Mechanisms).

While the vast majority of contemporary Chronobiological studies use laboratory regimes to study rhythms, together with late Prof Vijay Kumar Sharma, we started experiments using Drosophilids in an outdoor enclosure on our campus in Bangalore around 2010. Over the years our research has contributed towards understanding of how natural light and temperature cycles differentially shape behavioural patterns of multiple fly species (Prabhakaran et al., 2013, PLoS One; De, Varma et al., 2013, PNAS). In addition to seeing the acute response to naturally varying environmental features, we began a long-term experimental evolution study where we maintained Drosophila populations in a seminatural enclosure. As of now these populations have experienced daily and annual variations in light, temperature and possibly several other abiotic and biotic factors for approximately 200 generations.

We find unique changes in these populations in the phasing of eclosion rhythms in a season-specific manner (Dani and Sheeba 2022, Front Physiol).

Circadian clock-mediated rhythmicity is most evident for humans in their daily sleep-wake patterns and it is easy to recognize that some individuals in a population may have strikingly divergent preferences for sleep timing – 'morning /or lark' and 'evening / owl' types. Heritable variations of such preferences are referred to as chronotypes. Late Prof Vijay Sharma had initiated a set of populations of flies through long-term laboratory selection for divergent phasing of eclosion resulting in divergent chronotypes. In recent years we have continued to uncover new aspects of circadian clock organization among these chronotypes – studies of Abhilash Lakshman showed that the relative contributions of the theoretical A-B oscillators may have been altered among the early and late chronotypes and that the relative sensitivity to temperature may be altered (Abhilash et al., 2019; 2020a, 2020b, J Biol Rhythms). Additionally, by using a novel-light regime, Lakshman Abhilash showed that the extent of plasticity of the circadian clock neuronal circuit has also been changed among the chronotypes– the evening chronotypes exhibited reduced plasticity of amplitude of rhythms while showing high plasticity or phasing (Abhilash et al., 2020a, J Biol Rhythms).

Arijit Ghosh found that in addition to the changes in circadian entrainment properties, the 'early' chronotype flies have also evolved higher levels of 'masking', a phenomenon by which rhythmicity is generated by non-clock processes (Ghosh et al., 2021, J Biol Rhythms). Rhythmic events which are immediate responses to cycling environmental features have long been considered by chronobiologists merely as a phenomenon to be eliminated from experimental results as it can 'mask' the features driven by the circadian clock. However, in the real world, it can be expected that often such 'masked' rhythms co-evolve alongside true clock driven components and determine real-world phase of such rhythms. This is the first ever demonstration of the evolution of masking along with circadian clock driven features using replicate populations of D. melanogaster.

Abhilash Lakshman and Arijit Ghosh have each developed open-source analyses applications (RhythmicAlly - Abhilash and Sheeba 2020; VANESSA - Ghosh and Sheeba 2022 J Biol Rhythms). Both these applications have been extensively utilized and appreciated by the community for their comprehensive, user-friendly, and easy-accessibility features.

Caffeine disrupts the circadian clock in flies

Aishwarya Segu and Nisha N Kannan
Indian Institute of Science Education and Research, Thiruvananthapuram

Sleep is an essential resting phase, observed in most of the organisms. It is majorly regulated by the homeostatic processes and the circadian clock, which controls the depth and

timing of sleep respectively. Depth or amount of sleep defines sleep consolidation and is majorly mediated by the neurotransmitters and modulators. The fragmentation of sleep or broken sleep is due to the dysfunction of this pathway. Whereas, the circadian clock or the internal body clock regulates timing of sleep according to the environmental cyclic time cues. The interplay between both the homeostatic and the circadian process, orchestrates the sleep cycle and this has been well demonstrated using the two process sleep model.

Caffeine, a psycho-stimulant, induces wakefulness and thus affects the sleep cycle. Caffeine induces hyperactivity by increasing dopaminergic signaling. The effect of caffeine leading to sleep reduction is majorly due to this. These effects of caffeine on the depth of sleep are widely studied whereas, the impact of caffeine on the circadian rhythm is under explored. Owing to its wide use, side-effects and effects on sleep health it is thus salient to understand the effect of caffeine on the circadian timing system. Studies in the past have widely explored the behavioral effects of caffeine in mammalian systems including mice, rats and human beings. In our recent study we used *Drosophila* as our model organism to understand the effect of caffeine on sleep and circadian clock. We designed experiments to understand the effect of caffeine on sleep and circadian rhythm in an age and duration dependent manner. Age dependent caffeine treatment showed a significant increase in sleep fragmentation with increasing age indicating that caffeine might be affecting sleep fragmentation pathway. Our results for the first time show that caffeine leads to increased sleep fragmentation in an age dependent manner.

The next major question was to understand the duration dependent effects of caffeine. To examine this we had two cohorts of treatments with one short exposure and >10 days of prolonged caffeine exposure. Under short exposure we observed an overall decrease in sleep and contrastingly, in prolonged exposure we did not find any decrease in sleep indicating either tolerance or resistance to caffeine. Although, we did not find a decrease in sleep post prolonged exposure to caffeine this affected the sleep timing in flies indicating that circadian clock may be affected by prolonged caffeine treatment. To understand the impact of caffeine on the circadian rhythm we analysed the activity-rest rhythm under prolonged caffeine treatment. *Drosophila* exhibit a bimodal peak of activity corresponding to the morning and evening time respectively and under prolonged caffeine treatment we observed a decrease in the morning and evening activity. The circadian pacemaker neurons in the fly brain senses the time of the day, which ultimately reflects in increased activity during dawn and dusk. Caffeine flies indeed showed a decrease in their anticipatory activity and hence it is important to study whether caffeine impairs the circadian pacemaker neural connections.

These results led us to wonder if caffeine could affect the molecular circadian clock. The molecular core clock in *Drosophila* is well studied and is discovered to be mediated by negative transcriptional-translational feedback loop majorly consisting of the genes timeless and period and its transcriptional factors CLOCK and CYCLE. Upon prolonged caffeine treatment we observed a phase delay in transcript

oscillation of the timeless gene. To understand the effect of caffeine on the endogenous circadian clock we recorded locomotor activity rhythm under constant darkness in the presence of caffeine. Flies under prolonged caffeine treatment showed a less robust clock with either a delayed free running period or itled to complete abolition of the clock resulting in behavioral arrhythmicity.

Overall, our results recently published in Sleep Advances showed that caffeine, although being a wakefulness promoting small molecule, affects the key molecular component of the circadian clock. We depict that the duration of caffeine treatment plays a crucial role in the sleep and circadian rhythm. Short exposure to caffeine affects the sleep amount whereas prolonged treatment delays the free running period and causes arrhythmicity which could be due to effect on the molecular circadian pacemaker which needs to be validated in future.

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Grandfather clock

Neeti Badigannavar
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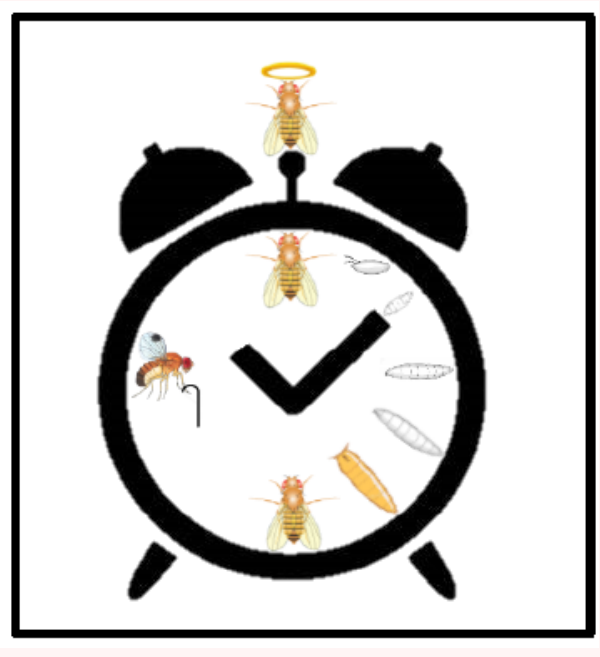
Aging is a natural process of growing old where the body approaches senescence and gradually losses the ability to perform various biological functions. Functions such as metabolism, sleep, activity, learning and many others are important for healthy living while their deterioration with increasing age can lead to severe health problems in the elderly [1]. Several theories have been proposed to explain this decay of bodily functions with age such as the genetic theory which involves telomeres and epigenetic factors. Some non-genetic theories suggest wear and tear of tissues, accumulation of reactive oxygen species (ROS) and other harmful substances within the body can also contribute [2]. Aging affects processes at all organizational levels such as cellular, tissue and systemic, and one among them is the circadian system. Circadian rhythms are cyclic phenomena with a period of 24 hours. These are the output of endogenous biological clocks which control various cyclic processes such as sleep-wake cycle, metabolism, temperature, activity/rest rhythms, hormone or neurotransmitter release, reproductive cycles etc., and can be synchronised by environmental cues [3, 4]. It will be helpful to study effects of aging on circadian clocks as they affect many aspects of overall physiology and behavior of organisms. Central clocks which reside in the brain, most notably the suprachiasmatic nucleus in mammals can either directly influence behavior by secreting hormones, neurotransmitters, through sleep-wake cycle; or indirectly by influencing or regulating peripheral clocks such as liver, kidneys, adrenal gland, prostrate and others. These peripheral

clocks are secondary oscillators which have their own cycles but are synchronized with the central clock [5, 6]. As the effects of aging on circadian clock can drastically impact health through both central and peripheral clocks, a better understanding of the changes in biological clocks themselves and their impact on physiology and behaviour throughout the aging process is of importance. This becomes even more relevant at a time when we have a rapid rise in the aging population (above 65 years) across the world and in our country.

Several models have been used to study the effects of aging on circadian clocks such as zebrafish (*Danio rerio*), flies (*Drosophila melanogaster*) and rodents (mice and rats). The fly model offers several advantages to conduct studies on the effects of age on circadian rhythms. It is easy to culture and has a short life span of 60-80 days and a well characterised circadian system with a variety of easily quantifiable behavioral rhythms such as activity-rest, eclosion, feeding and egglaying. Moreover, various life-history traits and rhythms can be tracked across age throughout its life-span [7].



The central clock of *Drosophila* comprises of ~150 clock neurons which are interconnected and control various rhythmic processes through their connections with other neurons via release of various neuropeptides and neurotransmitters. The ventrolateral neurons and dorsal lateral clock neurons majorly act as pacemakers for the locomotor activity rhythm [8]. The underlying molecular mechanism comprises of interlocked transcription-translation feedback loops with genes *period* (*per*), *timeless* (*tim*), *clock* (*clk*) and *cycle* (*cyc*) and their protein heterodimers playing crucial roles. Other major players include the neuropeptide Pigment Dispersing Factor (PDF) which is important for morning bout of locomotor activity and for setting the phase of the evening bout while the CRYPTOCHROME (CRY) provides light input to the core clock [9, 10].

Various studies have provided evidence for clock decay as the fly ages, at both behavioral and molecular level. Old flies show lengthening in the free running period of locomotor activity eventually leading to arrhythmia under constant conditions [11]. With age, more fragmented sleep with shorter sleep bouts, reduced sleep recovery after deprivation and decreased arousal threshold have also been reported [11, 12]. At the molecular level, decreased levels of *per* and *tim* mRNA levels as well as PER and TIM protein levels are seen. However, this was not observed for *clk/cyc* mRNA levels, showing that there was no difference in the levels of CLK/CYC proteins with increasing age. This suggests a weakening of the clock transcriptional-translational feedback loop, which also influences the various behavioral rhythms [13]. Additionally, in older flies, declining levels of PDF and CRY in head and retinal

cells is reported along with reduced photoreception. Interestingly, while in central pacemakers, lowered levels of PER and dampened oscillations have been observed, strong circadian protein oscillations persist in peripheral clocks like the fat bodies and the Malpighian tubules [14].

There have been speculations on the existence of bi-directional relationships between aging processes and circadian clocks. Some studies have shown evidence for decreased lifespan of certain circadian clock mutants. On the other hand, over-expression of certain clock proteins such as PDF and TIM have shown rescue of age-related behavioral arrhythmia and shortening of period in older flies [11,15]. However, these results are only suggestive and many aspects of such a relationship, if it exists, remain to be understood thus making it an open avenue for exploration.

Ernest Hemingway had once asked in his book 'The old man and the sea' that "Why do old men wake so early? Is it to have one day longer?", and it seems that the old man (or fly)'s circadian clock might hold some answers to that question.

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Dietary restriction for improved circadian rhythms during aging

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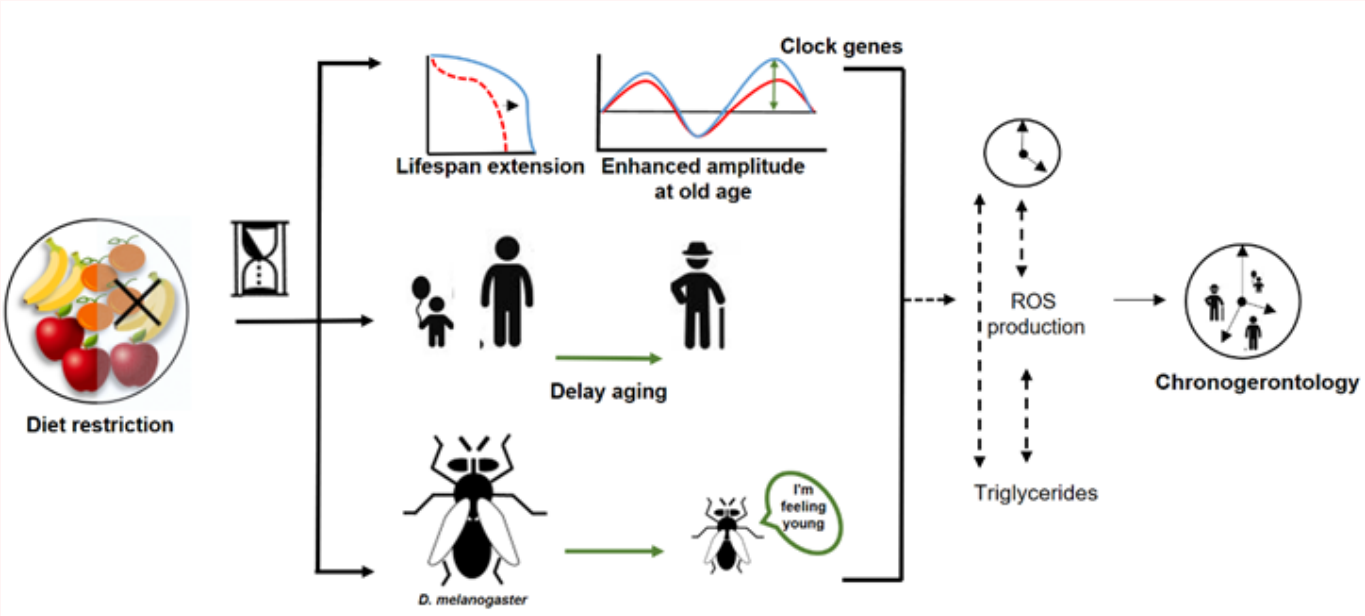
Diet is a pivotal factor that regulates a multitude of processes such as energy production, growth, metabolism, and organismal fitness, which ultimately influences the aging rate in most of the model organisms including fruit flies *Drosophila melanogaster* (Sun et. al., 2013). Studies have shown the role of nutrient components on lifespan extension, delayed aging (particularly via dietary restrictions- DR), and interconnections with circadian clocks. Since pre-adult development time is a life-history trait that is under the influence of circadian clocks and is correlated with the aging rate (Buck et. al., 2000), hence, under DR also, (low dietary protein or carbohydrate levels) it can be conceived that *Drosophila* pre-adult development might affect the aging rate of flies. Despite the nutrition compensation property of circadian rhythms, DR affects the locomotor activity levels/ bouts which are regulated via PAR domain protein 1 (*Pdp1*). *Pdp1*null mutants have shown reduced clock gene expression, while they fail to express clock genes Period (*per*), Timeless (*tim*), and PDF gene (Pigment Dispersing Factor) in the brain of *Drosophila* (Cyran et. al., 2003). Thus, the manifestation of maximum DR effects imperatively requires intact and functional circadian clocks (Katewa et al. 2016; Krittika & Yadav 2019).

Last decade's studies suggest that there are interconnections between circadian clocks and aging. Metabolism and circadian clocks are tightly intertwined, as the metabolism of lipids, glucose, and fatty acid is regulated by some of the circadian clock genes (Cermakian et. al., 2001; Ceriani et. al., 2002). Circadian clocks control the oxidative stress, cell cycle, cell death, and DNA damage response, which in turn regulate the organismal lifespan and are thus implicated in the control of aging and aging-associated pathologies (Costa & Ripperger, 2015; Khapre et. al., 2010); therefore, circadian clocks can be considered as one of the regulators of the aging process. While intact clocks can positively influence longevity and aging (Froy, 2011), circadian disruption through genetic ablation has been shown to reduce lifespan and bring about several pathophysiological changes (Yu & Weaver, 2010). Hence, strengthening the clock's role is required for the regulation of organismal physiology and the functioning of multiple signaling pathways (Costa & Ripperger, 2015).

Given that the circadian clock orchestrates various biological processes, disruption of circadian rhythms is associated with obesity, aging, and age-related pathologies (Reddy & 'Neill 2010; Froy & Miskin 2010). Additionally, under DR, circadian clocks are involved in the regulation of fat metabolism (Katewa et. al., 2016). Since triglyceride metabolism is conserved in mice as well as flies, further studies can be focused on the Sirtuin pathway and overexpression of *Sir2* in flies to escalate fat metabolism. Further, the circadian clock may adjust the feeding

patterns by anticipating the resource availability in an organism's ecological niche. It is thus not surprising that nutrients can also strongly entrain the peripheral clocks, especially those in adipose tissues and the liver (Gachon et al. 2004).

DR improves the circadian rhythmicity by enhancing the amplitude of various clock genes in the whole body rather than being limited to the brain of *Drosophila* arguing for an impact on peripheral clocks. Furthermore, circadian changes in genome-wide expression patterns resulted in an increased amplitude of circadian expression of many genes and proteins, suggesting that DR may enhance the function of the circadian clock and protect against the age-related decline of circadian clock function. In addition to this, DR is capable of influencing the mRNA expression levels of clock genes like *Per*, *Tim*, *Pdp1*, and *Vri* in fruit flies and also enhances the amplitude of cycling of *tim* (Katewa et. al., 2016). Hence, long-term DR feeding can be imposed along with intact circadian clock functioning to decipher the interconnection between the clock, reactive oxygen species (ROS) production, triglycerides, *etc*. which in turn might give better health and a holistic approach to understand the organismal physiology. Taken altogether, we can expect that the pattern of current research can be drastically transformed by implementing the "time-of-day" into account in all age-associated perturbations. Hence, a paradigm shift should be applied for the treatment of age-associated problems probably by initiating a sub-discipline 'chronogerontology', which might open a chance for understanding how diet restriction may enhance and protect against the age-related decline of circadian clock function.



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***Drosophila* as a screening platform for drugs which target the circadian system and sleep**

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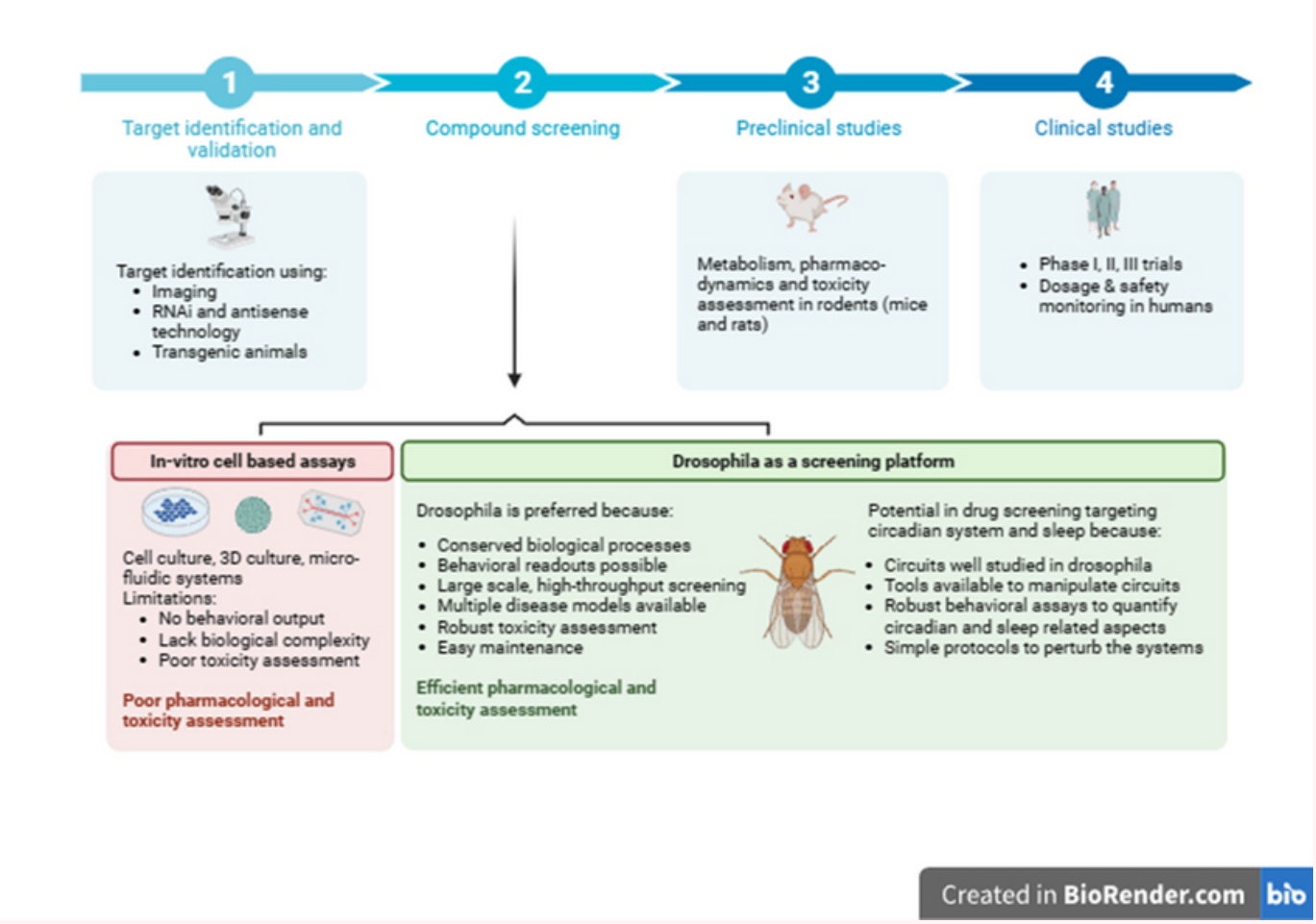
We all have taken a drug once in our life. A drug is a substance used to prevent or cure a disease. Considering the different diseases and ailments that humans are susceptible to, we cannot imagine our lives without these medicines. To provide effective and safe therapy for each disease, the pharmaceutical industry is constantly searching for new drug molecules. However, the process of drug discovery and development is complex. From the initial screening for potential drug candidates to its approval for use in the market, it takes around 10 years and costs about 2 billion US dollars.

The drug discovery process starts with identifying unmet needs related to disease and finding molecular targets involved in disease pathology. Targets involve enzymes, receptors, ion channels, or transporters whose modulation (activation/inhibition) can prevent or cure disease. After identifying the target, the search for molecules that can potentially bind to it begins. Thousands of molecules in chemical compound libraries are screened for their selectivity to the target, their activity, and toxicity via in-vitro cell-based assays, a process called drug screening. Only those compounds found to be safe and effective in the primary screen are further rigorously tested for their pharmacological and toxicity profile in animal models (mice, rats) and later in

humans. However, it is often the case that the majority of positive hits from *in-vitro* assays are later found to be ineffective or toxic in whole animal models, costing precious time and money. The most well-known example is that of the drug Thalidomide, which did not show any toxicity during in-vitro screening but caused havoc in humans. While the currently used cell-based methods such as cell lines, organoids, 3-D cultures, and microfluidic systems are vastly superior to previous cell culture systems, they continue to share major disadvantages. (a) These methods only provide molecular read-outs (as increase/decrease or activation/inhibition of target) but do not provide physiological/behavioral outputs. (b) Effects due to complex systemic biological interactions cannot be assessed and (c) Toxicity data is relevant to only particular cells and conditions and toxicity due to complex biological interactions/ due to involvement of multiple systems is lacking. These limitations bring the inefficiency in providing vigorous toxicity and efficacy data leading to the failure of molecules in later stages of drug development. Hence, there is a need for a screening platform at the initial stage of drug discovery that includes the biological complexities of a multi-cellular organism with simultaneous ease for rapidly extracting the behavior and toxicity-related data to obtain strong pharmacological and safety profiles of molecules.

What can be a better option than the common vinegar fly or fruit fly *Drosophila melanogaster*? It is perhaps the most extensively studied invertebrate with many conserved biological, physiological, and neurological properties as mammals. Around 75% of the genes associated with human diseases have functional homologs in flies, which suggests highly conserved disease pathways as humans. Flies provide additional advantages of screening a large number of compounds on large numbers of flies rapidly and cost-effectively compared to other more complex animal models. In addition, they can be procured and maintained easily at low cost as compared to cell lines and cultures. A wide variety of tools for genetic manipulation are available which are being used to model human diseases and for drug target identification. With flies it is also possible to study acute and chronic effect of molecule on physiology and toxicity. Additionally, flies also show robust behaviors and a variety of behavior assays are available to assess different physiological circuits. Hence, correlating the molecular information with the behavior will help make stronger conclusions related to the efficacy and toxicity of molecules. With all these advantages over the cell-based screening methods, flies can prove to be 'fruitful' in drug discovery.

Currently, well-established disease models of neurodegenerative conditions (such as Huntington's, Alzheimer's, Parkinson's disease, and Amyotrophic lateral sclerosis), diabetes, muscular dystrophy, and cancer are available. Hence, these models can directly be used for screening of neuroprotective, anti-diabetic, and anti-cancer medicines. Screening the molecules for circadian rhythms and sleep-wake cycle in flies holds tremendous promise. This is because the tools and techniques required to study circadian biology and sleep is well-established in flies and relatively large-scale, high-throughput assays can be performed. The circadian neuronal circuit is well-known



and neurons are well-characterized. Also available genetic manipulation tools allow us to manipulate (activate/inhibit) specific neurons in the circadian circuit in a spatio-temporal manner. Resulting behavioral change can be easily studied via various behavioral assays. Various aspects related to circadian rhythm and sleep such as jet lag, internal clock disruption and sleep deprivation can easily be studied since these conditions are easy to create in fly using simple protocols. Sleep in invertebrates has most of the hallmarks of vertebrate sleep such as circadian and homeostatic regulation, increased arousal thresholds and rebound sleep. *Drosophila* arousal tracking system (DART) allow us to study these sleep related aspects in fly. Different sleep stages are also currently being characterized in flies as that of mammals. We can also distinguish whether the sleep modulating compounds are affecting total sleep time or number of sleep episodes. Overall, these established knowledge and assays can directly be employed during drug screening for molecules affecting circadian system and sleep.

Upcoming events

International Symposium on Avian Endocrinology- 2024

March 17-22, 2024

CCS University, Meerut, UP, India

For more, visit: https://avianendoindia.com

or email: isaeindia24@gmail.com

2024 Annual Meeting of Society for Research on Biological Rhythms

May 18-22, 2024

San Juan, Puerto Rico

For more, visit: http://srbr.org

Sapporo Symposium on Biological Rhythms and Asian Forum on Chronobiology 2024

August 09-12, 2024

Hokkaido University

For more, visit: https://aschoff-honma.wixsite.com/ahmf/sapporo-symposium2022

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