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DAILY VARIATION OF BRAIN PROTEIN IN THE PRE-FRONTAL CORTEX OF ARVICANTHIS ANSORGEI

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ABSTRACT

Objective: To examine the daily variation of total protein contents in the pre-frontal cortex of the diurnal rodents, *Arvicanthis ansorgei*.

Study Design: Quasi-experimental study.

Place and Duration of Study: Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore Pakistan, form Jan to May 2023.

Methodology: Male young rats were forfeited at various zeitgeber times (ZT1, ZT5, ZT9, ZT13, ZT17, and ZT21), ZT0, and ZT12 characterizing lights on and off respectively. The brains were separated on ice and the pre-frontal cortex was isolated. The quantity of total protein contents was assessed using the Bradford test on the brain supernatants.

Results: The total brain protein contents in the prefrontal cortex of *Arvicanthis ansorgei* follow a circadian variation during the 24 hours of the day. Repeated measure ANOVA indicated a significant difference among all the six different time points, F (1,5)=595.476, $p<0.001$.

Conclusion: The first evidence that total protein contents alter at different times of the day in the pre-frontal cortex with a peak at ZT17 (5h after light off) and low at ZT1 (1h after light on) in diurnal rodents.

Keywords: Pre-frontal cortex, Zeitgeber Time (ZT), Circadian rhythms, total protein, Bradford assay, Arvicanthis ansorgei.

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INTRODUCTION

Circadian rhythms are oscillations occurring within twenty-four hours.[1] Circadian rhythms can be altered in their environment by external cues or stimuli called zeitgebers, the primary one of which is daylight.[2] Circadian rhythms have not been observed only in animals but also in other living beings.[3] Different physiological activities vary during the day and are at peak at one time and down at another point during the 24 hours. It has been confirmed by several studies on mammalian species that suprachiasmatic nucleus (SCN) which is a part of the hypothalamus in the brain is the circadian pacemaker.[4] It receives signals from the surroundings and delivers the fundamental clocking signals for coordinating the daily fluctuations in peripheral tissues.[5]

The brain's protein expression is fundamental to its function and is subject to daily rhythms, influenced by both intrinsic and extrinsic factors. The prefrontal cortex (PFC) plays an important role in cognitive functions such as decision-making, attention, and social behavior, making it a vital area of interest in understanding how protein fluctuations affect these processes. The *Arvicanthis ansorgei* (Nile grass rat) presents an excellent model for studying daily variations in brain protein expression due to its diurnal activity pattern, which closely resembles that of humans.

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Previous studies have shown that protein levels in various brain regions can fluctuate in response to circadian rhythms, environmental stimuli, and physiological demands. However, the daily variation of protein expression specifically in the prefrontal cortex of diurnally active species like *Arvicanthis ansorgei* remains poorly understood. By examining these variations, we can better understand how the PFC adapts to daily cognitive demands and how disruptions in these patterns might contribute to neurological disorders. This research aims to explore the temporal patterns of brain protein expression in the PFC of *Arvicanthis ansorgei* and its potential implications for cognitive function and circadian regulation.

Understanding the daily variation in protein expression in this region may provide insights into the mechanisms that underlie cognitive performance and neuroplasticity and offer a comparative framework for translational studies related to human neurological and psychiatric disorders.

The pre-frontal cortex (PFC) has been implicated in a wide variety of functions from language processing to executive functions. Execution of these functions is mediated as PFC consists of rich cortical and subcortical connections that are involved in initiating and maintaining specific behavior.[7] PFC has been also implicated in the formation of different types of memory and memory-related functions like short-term memory tasks, planning and decision making, delayed responding active problem solving, etc.[8]

Protein play an important role in the physiology and anatomy of the organ and system. So, there may be daily variation in functioning if there is a variation of protein in circadian fashion. Studies have shown circadian rhythmicity in short-term associative memory during light-dark periods.[9-12]

The objective of the this study was to investigate any circadian rhythm in total protein in the PFC of *Arvicanthis ansorgei*.

METHODOLOGY

This study was conducted in the department of Physiology of Institute of Molecular Biology and Biotechnology of University of Lahore. All the procedures were performed according to institutional guidelines that comply with national and international guidelines. Young male rats, *Arvicanthis ansorgei*, were acclimatized in 22-24°C (12:12 light-dark cycle) with ad libitum for 15 days. These rats were divided into six groups on the basis of zeitgeber time (ZT). Six subjects were kept in each group and subjects of each group were sacrificed at their specific zeitgeber time after euthanasia under CO2 at a concentration of 0.5 bars for a maximum of one minute. After euthanasia, the brains were quickly detached on ice, and the structure of PFC for each subject was removed in a typical extraction buffer. PFC homogenate after triturating with the buffer centrifugated at the speed of 4000 rpm for 20 minutes. Supernatants for the PFC were collected and frozen at -20°C till experiments. 3 µl of sample from the supernatant of the PFC were loaded into the respective cuvettes and each cuvette was loaded with 200 µl of Bradford reagent (Bradford Protein Assay, BIO-RAD USA). Samples and Bradford reagents were mixed properly together within the cuvettes. Each sample was allowed to be incubated at room temperature for five minutes. The absorbance of every sample at 595 nm was measured by using a UV-visible spectrophotometer.

RESULTS

The first time that the total brain protein contents in the prefrontal cortex of *Arvicanthis ansorgei* follow a circadian variation during the 24 hours of the day when animals were forfeited at six different zeitgeber times. These times included ZT1, ZT5, ZT9, ZT13, ZT17, and ZT21. Repeated measure ANOVA was used to find any meaningful difference at diverse time moments. For repeated measure ANOVA, Mauchly's test did not indicate any violation of sphericity, X2(14) = 8.547. Repeated measure ANOVA indicated a significant difference among all the six different time points, F (1, 5) =595.476, $p<0.001$. The mean, standard deviation, and lower and upper bound values of each zeitgeber time are shown in Table-I. The daily variation of protein contents in the pre-frontal cortex of *Arvicanthis ansorgei* at six different time points in Figure-I.

Table-I: Means, Standard Deviation, lower and upper bound values of total protein contents in the pre-frontal cortex of Arvicanthis ansorgei.

ZT	Mean ± SD	95% Confidence Interval		F	*p*-value
		Lower Bound	Upper Bound		
ZT1	1.367 ± 0.156	0.965	1.769	595.47	<0.001
ZT5	1.767 ± 0.189	1.281	2.253		
ZT9	1.482 ± 0.099	1.228	1.735		
ZT13	1.608 ± 0.150	1.223	1.994		
ZT17	2.122 ± 0.207	1.590	2.654		
ZT21	1.535 ± 0.105	1.265	1.805		

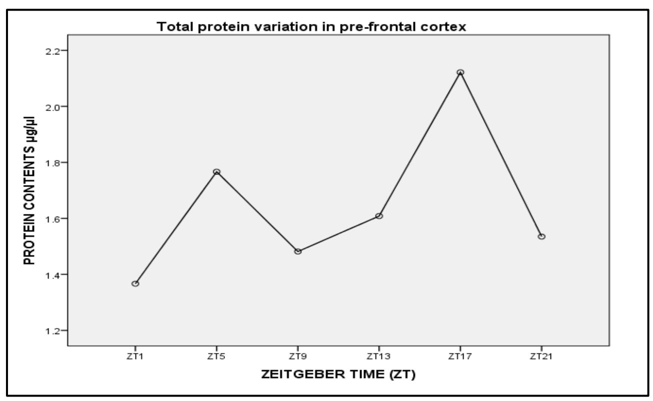

Figure-1: Total protein contents in pre-frontal cortex (µg/µl) of Arvicanthis ansorgei.

DISCUSSION

In this study, researchers observed the daily rhythmicity of total protein contents in the PFC of *Arvicanthis ansorgei* without any manipulation when subjects were sacrificed at six different time points during twenty four hours of the day. Our main results showed significant differences in total protein contents among different time points of the day. The peak of the total protein contents was observed at ZT17 (5 hours after lighting off) and low at ZT1 (1 hour after lighting off). We used *Arvicanthis Ansorgei* to study the circadian profile of the protein contents, as these animals are good diurnal subjects to study circadian rhythms.[13] We quantified total protein in the samples by using the Bradford assay which is considered an easy and quick method for measuring protein in solution and homogenates.[14]

Previous studies have shown circadian variation of different specific proteins; however, there is no data in the literature that shows daily variation of total protein in the brain. Few studies on brain proteins have shown circadian variations in specific parts of the brain, like an important brain-derived protein, brain-derived neurotrophic factor (BDNF) follows circadian rhythms in the suprachiasmatic nucleus.[15] Few studies also show that BDNF mRNA in the hippocampal subfields also follows circadian patterns in the hippocampus.[16,17] Some other important proteins like the mitogen-activated protein kinase (MAPK) and cyclic adenosine monophosphate (cAMP) proteins have very important roles in the strengthening of hippocampus-supported memory. It has been shown that extracellular synchronized kinase 1/2 (ERK1/2) and cAMP follow circadian rhythms in the hippocampus.[12]. compared to the dark era, the light period showed higher proteins. There is no data available on the total protein contents in the brain tissues. It is the first time that we investigated total protein contents in the PFC. The above studies show higher levels of specific proteins in the light period than the dark period is contrary to our data that show total brain protein in PFC is at peak in the dark period and vice versa.

It might be helpful to understand that at one specific zeitgeber time, a specific protein varies as compared to other zeitgeber times. This concept may be further helpful in designing the experiment with more specification and this may be helpful in pharmacological treatments at specific times of the day. It has been seen that anesthetics have different pharmacological effects at the molecular level.[18]

In addition, this circadian fashion of protein may be important for the normal functioning of physiological processes. Studies on various organisms have suggested that circadian rhythmicity plays an important role in the creation, steadiness, and recall of memories.[19] The peak of the protein levels during the 24 hours may reflect the peak of performance in specific processes and vice versa, as memory performance is observed higher in the light period than the dark period.[11].

CONCLUSION

Total protein contents in the *Arvicanthis Ansorgei* follow a circadian pattern during 24 hours of the day. The levels of protein contents were at peak at ZT17 and nadir at ZT1.

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Conflict of Interest: None.

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