INTRODUCTION

Locomotor activity is one of the major assessments that reflect individual animal gross behavior in animal research.\(^1,2\) Numerous research using locomotor activities have been performed to explore the changes of gross motor activities by experimental drugs.\(^3\) In addition, using the strong association between locomotor activity and sleep-wake state, there were studies to investigate sleep-wake states.\(^4\) Furthermore, locomotor activities can be analyzed in a perspective of circadian rest-activity rhythm.\(^5,6\) Even though locomotor activities show the overall results of individual behavior that respond to the external situation at that moment, locomotor activities are bound to be under the gross influence of day and night. For this reason, locomotor activities can show circadian rest-activity rhythm that is one of the indices of circadian rhythms.\(^7\)

In the area of research of circadian rhythm, several studies have used the circadian indicies of rest-activity rhythm that involved locomotor activities. Among various methods for analysis of circadian rest-activity rhythm, cosinor analysis is a common analytic methodology in circadian rhythm research areas using locomotor activity.\(^8\) Many studies have widely used cosinor analysis to analyze circadian rest-activity. However, specific analyzing methods such as sampling rates and data processing in raw data have been performed in various way.\(^9\) In previous studies, sampling rates have been used at various time intervals from one minute to one hour. One minute can be used as a basic sampling rate.\(^10,11\) Sampling rates in multiples of three, such as 3 minutes\(^12,13\) or 6 minutes\(^14\) can be widely used, because it can be easily interpreted visually considering that a day is 24 hours. On the other hand, there are common cases where it is summarized in multiples of five, such...
as 5 minutes, 10 minutes, or 15 minutes. Meanwhile, raw data may be processed in various methods, such as data summation, data average, or data moving average.

Although cosinor analysis is performed in various ways in each study, it is necessary to provide sufficient rationality for these specific methods. However, due to several reasons, there are many cases that are used without reasonable evidence. Although high-resolution information has recently been obtained due to the development of technologies for measuring locomotor activity, there are some opinions that such high-resolution information may not necessarily be good. It depends on the research purpose, but in some studies, high throughput may act as noise. Therefore, an appropriate sampling rate is required according to the research purpose. As such, an optimal analysis method needs to be presented, and it is necessary to explore the relationship between these methods in order to compare existing studies. However, the difference among these data processing methods is unknown yet, and there was a lack of evidence for optimal analysis of circadian rest-activity rhythm. Therefore, we aimed to investigate the optimal strategy about the data processing method and the appropriate sampling rate for cosinor analysis in mice through a mathematical simulation according to the time block of data processing.

METHODS

Study subjects and procedure
Twenty male ICR mice (age: 4–5 weeks, weight: 21–27 g; Koatech, Pyeongtaek, Republic of Korea) were used in this study. The subjects were allowed to drink water and eat food freely. The temperature and humidity of the laboratory environment were maintained at 20°C±1°C and 40%–60%, respectively. The mice were acclimatized to the laboratory environment for a week. After the acclimatization period, the locomotor activities of the animals in homecages were collected every second by infrared red motion detectors for 24 hours. The illumination was controlled at the 12 h light–12 h dark cycle by an illumination control system (iW Blast Powerecore/Colorplay 3/Data Enabler Pro; Philips, Burlington, MA, USA). The lights were set to turn off at 5 P.M. and turn on at 5 A.M. The Animal Experiment Ethics Committee of Pusan National University Hospital approved this study (IRB number: PNUH-2017-118).

Assessment of homecage locomotor activities
The locomotor activities in homecages were measured by the MLog system (Biobserv Inc., Bonn, Germany). The MLog system installed in homecages detects the free locomotor activity using infrared motion sensors. The MLog system data were collected every second. If there was no activity, it was recorded as zero, and more activities were indicated by higher data values.

Analysis and mathematics simulation
Circadian rest-activity rhythms were analyzed by cosinor analysis. Cosinor analysis is a type of analysis that finds an optimal cosine curve to show the least square of the difference between the fitted cosine curve and the original raw data. Cosinor analysis estimates a circadian rhythm for 24 hours through a zero-amplitude test. The null hypothesis of zero-amplitude is that the amplitude is zero. Through cosinor analysis, circadian indices such as the amplitude, MESOR, and acrophase were calculated.

In order to find the most optimal time block for cosinor analysis, mathematical simulations were performed as explained in Figure 1. According to the increase in processing time per second in data summation, data average, and data moving average, the changes of circadian indices were simulated. When simulating the changes of circadian indices, the F and p values were calculated by zero-amplitude test to verify the accuracy of the values estimated by cosinor analysis. Cosine analysis, zero-amplitude test, and mathematical simulation were performed using MATLAB R2020b (MathWorks, Data acquisition Data processing Data analysis

Infrared locomotion detector (MLog system)

1. Data summation
2. Data average
3. Data moving average

Cosinor analysis

Figure 1. The schematic process to analyze circadian rest-activity rhythm according to the methods of data processing using locomotor activities.
Natick, MA, USA). Two values estimated by data average and data moving average were compared by intraclass correlation (ICC) and Pearson’s correlation by IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA), with a statistical significance level of less than 0.05.

RESULTS

Characteristics of estimated circadian rest-activity rhythms according to the methods of data processing

The estimated values of circadian rest-activity rhythm by data summation, data average, and data moving average are shown in Table 1. When using data summation, the estimated values gradually increased according to size of the time block. Meanwhile, the estimated values by both data average and data moving average were similar independent of time block. Based on the values estimated by data moving average, the amplitude was 3.631 (±1.137), MESOR 4.918 (±1.370), and acrophase -1.561 (±0.402), respectively.

Estimation of circadian rest-activity rhythm using data summation

When using data summation, the estimates of amplitude and MESOR gradually increased (Figure 2A). The acrophase showed a small variance within about 6,000 seconds, but the values were gradually increased according to the data processing time after 6,000 seconds. The F and p values in zero-amplitude test were statistically significant within about 800 seconds, but the significances were different depending on the subjects after 800 seconds.

Estimation of circadian rest-activity rhythm using data average

When using data averaging, the values of amplitude, MESOR, and acrophase were estimated (Figure 2B). Like the results by method of data summation, the estimated values showed a small variance within about 6,000 seconds, but the variance was increased gradually according to the increase of data processing time. Furthermore, the F and p values in zero-amplitude test were statistically significant within about 800 seconds like the results by the method of data summation.

Table 1. Circadian rest-activity rhythms estimated by cosinor analysis using raw data per one second in 20 mice

| Circadian index | Estimated value      |
|-----------------|----------------------|
| Amplitude       | 3.631 (±1.137)       |
| MESOR           | 4.918 (±1.370)       |
| Acrophase       | -1.561 (±0.402)      |

Data were shown as mean values (±standard deviation).

Figure 2. Mathematical simulation of circadian rest-activity rhythm analysis using cosinor analysis according to methods of data processing such as summation (A), average (B), and moving average (C). Cosinor analysis estimated the amplitude, MESOR, acrophase through cosine fitting based on observed data. These estimations were statistically tested by zero-amplitude test. Changes of estimated circadian parameters by data summation (A), data average (B), data moving average (C) were shown according to data processing time, respectively. Red line means p value 0.05 as a statistical significance.
Estimation of circadian rest-activity rhythm using data moving average

When using data moving average, the estimates of amplitude, MESOR, and acrophase were almost constant values within the ranges of all data processing time (Figure 2C). The F values gradually increased according to the increase of data processing time, and the p-values in zero-amplitude test were significant in all ranges from 1 to 21,600 seconds.

Comparison of circadian rest-activity indices using the methods of data average and data moving average

The values of circadian rest-activity rhythm estimated by cosinor analysis using data average and data moving average at the 800-second data processing were compared (Figure 3). These estimated values were compared by ICC and Pearson’s correlation. ICC analysis significantly showed a high concordance rate between two values estimated by data average and data moving average (800-second data processing: amplitude ICC=1.000 [p<0.001], MESOR ICC=0.912 [p<0.001], acrophase ICC=0.999 [p<0.001]). Additionally, there were highly significant correlations between two values estimated by data average and data moving average (800-second data processing: amplitude r=1.000 [p<0.001], MESOR r=0.838 [p<0.001], acrophase r=1.000 [p<0.001]).

DISCUSSION

This study performed mathematical simulations to find the optimal methods of cosinor analysis for studying circadian rest-activity rhythms. As shown in Figure 2C, the method of data moving average showed well-fitted cosine curves independent of the time blocks. As the data processing time with data moving average increases, the statistical power was increased. Given that the data moving average reflects the trend of data according to time, the increase of statistical power according to the increase of data processing time can be sufficiently expected. These findings suggest that the cosinor analysis by using data moving average would be an optimal method that could estimate the constant results for an analysis of circadian rest-activity rhythm regardless of data processing time.

In contrast, when using the data summation method, the amplitude or MESOR was inevitably increased as the time block increases as shown in Figure 2A. For this reason, when the circadian parameters were estimated by using data summation, the values of amplitude or MESOR analysis except acrophase cannot be absolutely compared with those by different time blocks. Meanwhile, when using one of data averages, amplitude, MESOR, and acrophase were estimated properly within 800 seconds as shown in Figure 2B. However, as the time block elongates, the variation of the estimated value tended to increase. Locomotor activities can reflect the ultradian rhythms in each mouse from several minutes to several hours except circadian rhythms.26,27 These ultradian rhythms which might be different in each mouse according to their dopaminergic states can hinder or distort fitting cosine curve around 24 hours.28 In this study, some results of cosine fitting after about 800 seconds did not show statistical significance. These different results of cosinor analysis might be attributed to individual ultradian rhythms of mice as previously noted. Thus, in order to avoid the influence of ultradian rhythms and properly estimate parameters of circadian rest-activity rhythm, a shorter time block within 800 seconds may be desirable. In addition, this study showed high concordance rate and high correlation between two values estimated by using data aver-
Analysis of Rest-Activity Rhythm

age and data moving average with 800-second time block. If a researcher wants to use the method of data average, the optimal time block for analysis would be the range within 800 seconds.

There are some limitations in this study. Firstly, this study only simulated the cosinor analysis for analyzing circadian rest-activity rhythm. Even though cosinor analysis is widely used for circadian rhythm analysis, other analyzing methods for circadian rest-activity rhythm can be used. Thus, these optimal methods on data processing can be adapted when using cosinor analysis. Moreover, this study tested three common methods such as data summation, data average, and data moving average, therefore it is possible that there could be a more optimal method. Furthermore, the analyses in this study were only performed based on the locomotor activities that an optimal strategy for data processing with other circadian indices such as temperature, melatonin, or cortisol should be performed in the future.

Nevertheless, this study suggests the optimal method for analysis of circadian rest-activity rhythm using cosinor analysis. This method can be effectively utilized to analyze circadian rest-activity rhythm using daily locomotor activities in mice. Additionally, when comparing the values estimated by different methods like data average and data moving average, these findings from this study can be helpful to integrate the results of studies using various data processing methods.

Locomotor activity is one of the important characteristics that reflects circadian rhythms. Through using cosinor analysis, the circadian indices such as amplitude, MESOR, or acrophase can be effectively estimated. These simulations suggested that data moving average would be an optimal method for data processing of cosinor analysis. Also, this study showed that the data average within 800 seconds of data processing time might be adaptable. These results can serve as the basic information for research of circadian rhythm using locomotor activities.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Jung Hyun Lee, Eunsoo Moon. Data curation: Jeonghyun Park. Formal analysis: Min Yoon. Investigation: Jung Hyun Lee, Eunsoo Moon. Methodology: Eunsoo Moon, Min Yoon. Project administration: Jung Hyun Lee, Eunsoo Moon. Resources: Jung Hyun Lee, Eunsoo Moon, Jeonghyun Park. Supervision: Eunsoo Moon. Validation: Chi Eun Oh, Yoo Rha Hong. Visualization: Jung Hyun Lee, Eunsoo Moon. Writing—original draft: Jung Hyun Lee, Eunsoo Moon. Writing—review & editing: Jeonghyun Park, Chi Eun Oh, Yoo Rha Hong, Min Yoon.

ORCID iDs

Jung Hyun Lee https://orcid.org/0000-0002-0496-9826
Eunsoo Moon https://orcid.org/0000-0002-8863-3413
Jeonghyun Park https://orcid.org/0000-0003-1050-212X
Chi Eun Oh https://orcid.org/0000-0002-0439-8170
Yoo Rha Hong https://orcid.org/0000-0002-7673-070X
Min Yoon https://orcid.org/0000-0002-6124-9163

Funding Statement

None

REFERENCES

1. Mony TJ, Lee JW, Dreyfus C, DiCicco-Bloom E, Lee HJ, Valproic acid exposure during early postnatal gliogenesis leads to autistic-like behaviors in rats. Clin Psychopharmacol Neurosci 2016;14:338-344.
2. Sui ZY, Chae HJ, Huang GB, Zhao T, Shrestha Muna S, Chung YC. Effects of chronic mild stress in female bax inhibitor–1–gene knockout mice. Clin Psychopharmacol Neurosci 2012;10:155-162.
3. Mihrara T, Kikuchi T, Kamiya Y, Koga M, Uchimoto K, Kuraushii K, et al. Day or night administration of ketamine and pentobarbital differentially affect circadian rhythms of pineal melatonin secretion and locomotor activity in rats. Anesth Analg 2012;115:805-813.
4. Park J, Jung MS, Moon E, Lim HJ, Oh CE, Lee JH. Prediction of locomotor activity by infrared motion detector on sleep-wake state in mice. Clin Psychopharmacol Neurosci 2021;19:303-312.
5. Hashinaga T, Wada N, Otabe S, Yuan X, Kuriya K, Kakino S, et al. Modulation by adiponectin of circadian clock rhythmicity in model mice for metabolic syndrome. Endocrin J 2013;60:483-492.
6. Oyebami O, Collins HM, Pardon MC, Ebling FJP, Heery DM, Moran PM. Abnormal clock gene expression and locomotor activity rhythms in two month-old female APPSw/PS1dE9 mice. Curr Alzheimer Res 2017;14:850-860.
7. Eckel-Mahan K, Sassone-Corsi P. Phenotyping circadian rhythms in mice. Curr Protoc Mouse Biol 2015;5:271-281.
8. Refinetti R, Lissen GC, Halberg F. Procedures for numerical analysis of circadian rhythms. Biol Rhythm Res 2007;38:275-325.
9. Portaluppi F, Tootiy F, Smolensky MH. Ethical and methodological standards for laboratory and medical biological rhythm research. Chronobiol Int 2008;25:999-1016.
10. Miyazaki K, Itoh N, Ohyama S, Kadota K, Oishi K. Continuous exposure to a novel stressor based on water aversion induces abnormal circadian locomotor rhythms and sleep-wake cycles in mice. PLoS One 2013;8:e55452.
11. Satoh Y, Kawai H, Kido N, Kawashima Y, Mitsumoto A. Time-restricted feeding entrains daily rhythms of energy metabolism in mice. Am J Physiol Regul Integr Comp Physiol 2006;290:R1276-R1283.
12. Li Y, Liu Y, Jiang Z, Guan J, Yi G, Cheng S, et al. Behavioral change related to Wenchuan devastating earthquake in mice. Bioelectromagnetics 2009;30:613-620.
13. Fenoglio-Simeone KA, Wilke JC, Milligan HL, Allen CN, Rho JM, Maganti RK. Ketogenic diet treatment abolishes seizure periodicity and improves diurnal rhythmicity in epileptic Kcnal-null mice. Epilepsia 2009;50:2027-2034.
14. Palmisano BT, Stafford JM, Pendergast JS. High-fat feeding does not disrupt daily rhythms in female mice because of protection by ovarian hormones. Front Endocrinol (Lausanne) 2017;8:44.
15. Sanchez-Alavez M, Alboni S, Conti B. Sex- and age-specific differences in core body temperature of C57Bl/6 mice. Age (Dordr) 2011;33:89-99.
16. Oike H, Nagai K, Fukushima T, Ishida N, Kobori M. High-salt diet advances molecular circadian rhythms in mouse peripheral tissues. Biochem Biophys Res Commun 2010;402:7-13.
17. Sasaki T, Numano R, Yokota-Hashimoto H, Matsu S, Kimura N, Takeu...
chi H, et al. A central-acting connexin inhibitor, INI-0602, prevents high-fat diet-induced feeding pattern disturbances and obesity in mice. Mol Brain 2018;11:28.
18. Mouralidarane A, Soeda J, Sugden D, Bocianowska A, Carter R, Ray S, et al. Maternal obesity programs offspring non-alcoholic fatty liver disease through disruption of 24-h rhythms in mice. Int J Obes (Lond) 2015;39:1339-1348.
19. Takasu NN, Kurosawa G, Tskada IT, Mochizuki A, Todo T, Nakamura W. Circadian regulation of food-anticipatory activity in molecular clock-deficient mice. PLoS One 2012;7:e48892.
20. Rozov SV, Porkka-Heiskanen T, Panula P. On the role of histamine receptors in the regulation of circadian rhythms. PLoS One 2015;10:e0144694.
21. Barca-Mayo O, Pons-Espinal M, Follert P, Armirotti A, Berdondini L, De Pietri Tonelli D. Astrocyte deletion of Bmal1 alters daily locomotor activity and cognitive functions via GABA signalling. Nat Commun 2017;8:14336.
22. Zhou P, Werner JH, Lee D, Sheppard AD, Liangpunsakul S, Duffield GE. Dissociation between diurnal cycles in locomotor activity, feeding behavior and hepatic PERIOD2 expression in chronic alcohol-fed mice. Alcohol 2015;49:399-408.
23. Ikeda E, Matsunaga N, Kakimoto K, Hamamura K, Hayashi A, Koyanagi S, et al. Molecular mechanism regulating 24-hour rhythm of dopamine D3 receptor expression in mouse ventral striatum. Mol Pharmacol 2013;83:959-967.
24. Bains RS, Wells S, Sillito RR, Armstrong JD, Cater HL, Banks G, et al. Assessing mouse behaviour throughout the light/dark cycle using automated in-cage analysis tools. J Neurosci Methods 2018;300:37-47.
25. Cornelissen G. Cosinor-based rhythmometry. Theor Biol Med Model 2014;11:16.
26. Storch C, Höhne A, Holboer F, Ohl F. Activity patterns as a correlate for sleep-wake behaviour in mice. J Neurosci Methods 2004;133:173-179.
27. Blum ID, Zhu L, Moquin L, Kokoeva MV, Gratton A, Giros B, et al. A highly tunable dopaminergic oscillator generates ultradian rhythms of behavioral arousal. Elife 2014;3:e05105.
28. Prendergast BJ, Cisse YM, Cable EJ, Zucker I. Dissociation of ultradian and circadian phenotypes in female and male Siberian hamsters. J Biol Rhythms 2012;27:287-298.