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Ultradian rest-activity rhythms induced by quinpirole in mice using wavelet analysis

ABSTRACT

Objective: Ultradian rhythms are biological rhythms with periods of a few seconds to a few hours. Along with circadian rhythms, ultradian rhythms influence human physiology. However, such rhythms have not been studied as intensively as circadian rhythms. In this study, our aim was to identify ultradian rest-activity rhythms induced by the dopamine D2 and D3 agonist quinpirole in mice using continuous wavelet analysis.

Methods: We used ten mice from the Institute of Cancer Research. Quinpirole was administered at a dose of 0.5 mg/kg. We assessed free rest-activity using infrared detectors and conducted wavelet analysis to measure the period and its variation. We also used the paired t-test to compare ultradian rhythm patterns.

Results: Quinpirole did not significantly change total 24-hour locomotor activity (p=0.065). However, it significantly increased locomotor activity during the dark phase (p=0.001) and decreased it during the light phase (p=0.016). In the continuous wavelet transform analysis, the mean period was 5.618 hours before quinpirole injection and 4.523 hours after injection. The period showed a significant decrease (p=0.040), while the variation remained relatively consistent (p=0.871) before and after quinpirole injection.

Conclusion: This study demonstrated ultradian rest-activity rhythms induced by quinpirole using wavelet analysis. Quinpirole-induced ultradian rhythms exhibited rapid oscillations with shortened periods and increased activity during the dark phase. To better understand these changes in ultradian rhythms caused by quinpirole, it is essential to compare them with the effects of other psychopharmacological agents. Furthermore, investigating the pharmacological impact on ultradian rest-activity rhythms may have valuable applications in clinical studies.

KEY WORDS: quinpirole, dopamine, locomotion, ultradian rhythm, circadian rhythm, wavelet analysis

Introduction

Biological rhythms are oscillations in organisms controlled by internal biological processes, each characterized by specific periods [1)]. These rhythms are evident in the physiology and behavior of organisms, with the circadian rhythm being one of the most commonly studied [2)]. Circadian rhythm is known as an important topic related to physical and mental illnesses, and it has an impact on the outcomes of psychiatric treatments [3-5)]. Therefore, it has become a target for medical treatment [6, 7)]. Besides the circadian rhythm, there are even shorter rhythms known as ultradian rhythms. These rhythms play critical roles in mammalian physiology, including functions such as thermoregulation, hormonal secretion, and rest-activity rhythm, much like the circadian rhythm. For instance, rats exhibit an ultradian oscillation of approximately two hours in core and organ temperature [8)]. Another study demonstrated that in constant light, the body temperature of male Wistar rats oscillated with a 3-5 hour period [9)]. Hormone secretions also exhibit ultradian rhythms. Evidence shows that in male rats, growth hormone is released with an ultradian rhythm of 3.3 hours [10)]. Ultradian patterns with periods of 3-5 hours were observed in locomotor activities in 11 distinct mouse strains [11)]. These examples demonstrate the involvement of ultradian rhythms in various biological processes, emphasizing their importance.

As mentioned earlier, ultradian rhythms are biological rhythms with recurring periods within a 24-hour cycle, and their importance is being emphasized. However, studies on ultradian rhythms are not as extensive as those on circadian rhythms. Locomotor activity refers to movement from one place to another [12)] and is a common method for analyzing circadian functions in animal studies [13)]. In one of our previous studies, we observed the locomotor activities of Institute Cancer Research (ICR) mice using an infrared detector monitoring system,

which focused solely on the circadian perspective and did not involve the use of any agents [14]. Furthermore, a study on C57BL/6 mice observed ultradian rhythms using a dopamine antagonist, haloperidol, and reported that when the animals consumed water with the agent, their ultradian rhythm shortened [15].

However, there is a lack of studies that have observed changes in locomotor activities in terms of ultradian rhythms. Understanding the alterations in ultradian rhythms is crucial, but finding the appropriate analysis method can be challenging. Cosinor analysis or cosine fitting analysis, which utilizes the least squares method for predictable rhythms, can be used to analyze biological rhythms [16, 17]. Also, Fourier transform, which decomposes functions into frequency components, could be utilized to analyze various rhythms. However, both cosinor analysis and Fourier transform have limitations when it comes to analyzing the variable periods of ultradian rhythms that may change over time. Recently, wavelet transform has been employed to analyze ultradian rhythms, which have periods within 24 hours [18, 19]. Wavelet transform is a mathematical tool used to overcome the limitations of Fourier transform [18, 20]. It analyzes datasets with constantly changing parameters in various scales, such as signals with frequency changes over time [21]. Therefore, wavelet transform is a suitable method for analyzing ultradian rhythms, and it has been used in recent studies [15, 22].

Previous study suggests that quinpirole can be a drug that mediated locomotor activity [23]. However, that study only analyzed locomotor activities with 12- or 24-hour summations based on the light-dark phase, and we didn't analyze locomotor activities from the perspective of ultradian rhythms. Therefore, our goal in this study was to explore the impact of the dopamine

agonist, quinpirole, on the period and variation of ultradian rhythms in mouse locomotor activity using wavelet analysis for observing rhythm trends over time.

Method

Subjects

To investigate the effects of quinpirole on locomotor activities and analyze ultradian rhythms, we reviewed previous experimental data [23]. This study used 10 male Institute Cancer Research (ICR) mice (weight 34-38g, age 5-6 weeks), identical to those in the previous study. While the previous study only explored circadian rest-activity rhythms, this study newly analyzed locomotor activities from the perspective of ultradian rhythms. As mentioned in the previous study, the animals were individually placed in cages under constant conditions with a temperature (20-25°C) and humidity (40-60%). They were provided with free access to food and water. A lighting control system (iW Blast Powercore/Colorplay3/Data Enabler Pro; Philips, Burlington, MA, USA) was used to maintain a 12:12 light-dark cycle (light phases: lights on from 5 PM to 5 AM, dark phases: lights off from 5 AM to 5 PM). This study was approved by the Institutional Animal Care and Use Committee at Pusan National University Hospital (PNUH-2017-018).

Experimental Procedure and Measurements

The mice were given a one-week accommodation period to adapt to the new environment after being placed individually in cages. Subsequently, locomotor activity data were collected using infrared motion detectors. After observing baseline locomotor activities for 2 days, quinpirole 0.5 mg/kg, which revealed a significant effect on open field test [24], was administered intraperitoneally at the beginning of the dark phase at 5 PM. Locomotor activities were then observed for 2 days after quinpirole administration.

Locomotor activity

The Mlog system (Biobserve Inc., Bonn, Germany), which utilizes infrared motion detectors, was employed to measure the animals' locomotor activities. Each detector was attached to individual home-cages and recorded the animals' movements continuously, with measurements taken every second.

Data Analysis

Locomotor activities were analyzed using continuous wavelet transform to determine the periods and standard deviations of ultradian rest-activity rhythms. To compare the effect of quinpirole administration on these rhythms, the data were divided into two sets: two days before quinpirole injection and two days after quinpirole injection. These data sets were then preprocessed using a moving average with an 870-second interval to optimize the analysis of rest-activity rhythms, following the methodology of a previous study [25]. The processed data sets were used to calculate the average and standard deviation of potential dominant ultradian periods using continuous wavelet transform for each animal. The standard deviation of the period represents the degree of variation in ultradian rest-activity rhythms. The moving average process and continuous wavelet transform were performed using MATLAB R2022 (MathWorks, Natick, MA, USA). To compare the periods and standard deviations of ultradian rest-activity rhythms before and after quinpirole administration, paired t-tests were conducted using IBM SPSS version 22.0 (IBM Co, Armonk, NY, USA).

Results

Table 1 showed locomotor activities before and after quinpirole administration. When the total locomotor activities from each day were summed and compared, there was no significant difference after quinpirole administration (t = -1.968, p = 0.064). However, there was a significant decrease in activity during the light phase sum, which represents the summation of locomotor activities during the light phases after quinpirole injection (t = 4.692, p = 0.001). Conversely, a significant increase in locomotor activities was observed in the dark phases, as indicated by the dark phase sum (t = -2.947, p = 0.016).

The continuous wavelet transform, following the moving average process, revealed a significant decrease in the mean period after quinpirole administration (t = 2.399, p = 0.040), while there was no significant change in the variation of the period induced by quinpirole (t = -0.168, p = 0.871) (see Table 1). On average, the period before quinpirole administration was 5.618 hours, but it shortened to 4.523 hours after the injections, as illustrated in Figure 1. The heatmaps generated from the continuous wavelet transform (Figure 2) displayed individual ultradian rest-activity rhythms before and after quinpirole administration. The periods of ultradian rest-activity rhythms before the quinpirole injections ranged from 4.090 hours to 5.850 hours, while after the injections, they ranged from 3.310 hours to 5.680 hours. The variations in period before the injections ranged from 1.340 to 3.140 hours, whereas after the injections, they ranged from 2.090 to 2.650 hours.

Discussion

In this study, we observed changes in ultradian rest-activity rhythms induced by a dopamine D2 and D3 agonist. The continuous wavelet transform analysis revealed that the ultradian rhythms had periods of approximately 4-5 hours at baseline, which aligns with findings from a previous study [15]. This study found that quinpirole injection decreased the mean period of ultradian rhythms and increased locomotor activities during the dark phase while decreasing them during the light phase [23]. When integrating the effects on ultradian and circadian rhythms, quinpirole may induce rapid oscillations with shortened periods and varying amplitudes between day and night as represented in Figure 1 [23].

Dopamine plays a significant role in both ultradian and circadian rest-activity rhythms. A study reported that dopamine-increasing agents, such as methamphetamine, induced longer periods in ultradian rest-activity rhythms [22]. Methamphetamine increased total locomotor activities and lengthened the circadian rhythm period. The increase in dopamine levels caused by methamphetamine might lead to longer periods in both ultradian and circadian rhythms [15, 22]. In contrast, haloperidol, a dopamine D2 receptor antagonist, shortened the ultradian locomotor period [15]. Furthermore, clinical observations have reported that haloperidol disrupts circadian rhythms and decreased daytime activity [26, 27]. Thus, dopaminergic agents have important effects on ultradian and circadian rhythms. Meanwhile, our findings showed that the dopamine D2 and D3 agonist shortened the period of ultradian rest-activity rhythms. In contrast to methamphetamine or haloperidol, quinpirole reduced the ultradian rhythm period but appeared to enhance the circadian rhythm. These results imply that locomotor activity can be affected distinctly based on the properties of dopaminergic agents. Previous studies have shown that quinpirole has a biphasic effect on locomotor activities, contrary to general

expectations that a dopamine D2 and D3 agonist might simply increase locomotor activities [24, 28]. This unique effect of quinpirole has previously attracted the attention of researchers. Our previous study demonstrated that quinpirole initially suppressed mouse locomotor activity but activated it after 30 minutes in the open field test [24]. Especially, low-dose quinpirole administration (0.5 mg/kg) decreased locomotor activities, while high-dose administration increased activities in the open field test [24]. Quinpirole is thought to show biphasic effects on locomotor activities through presynaptic inhibitory autoreceptors [24, 29]. The biphasic effect of quinpirole may have the dopamine related ultradian rhythms. That is, the biphasic effect is likely to have shortened the period of the ultradian rhythm.

Nevertheless, this study has several limitations. Firstly, the sample size used in the study is relatively small, which limits the generalizability of the effects of quinpirole. Larger sample sizes are needed. Secondly, because quinpirole was only administered once, the long-term effects of the drug could not be explored. It would be important to administer quinpirole multiple times or repeatedly to observe long-term effects. Thirdly, the study did not use different doses of quinpirole. Given the different effects that different doses of quinpirole can have on motor activity, this aspect should be explored in future studies [13].

In conclusion, we analyzed quinpirole-induced locomotor activity using continuous wavelet transform. The results showed an ultradian rest-activity rhythm of approximately 4 to 5 hours, consistent with previous studies. Interestingly, quinpirole shortened the period of the ultradian rhythm, indicating rapid oscillation. These findings suggest the need to investigate whether other pharmacological agents can influence ultradian rest-activity rhythms as well as circadian

rhythms. Additionally, future clinical studies, based on the results of animal studies, could be meaningful to understand the pharmacological effects on ultradian rest-activity rhythms.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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Table 1. Comparison of locomotor activities and the ultradian rest-activity rhythm before and after quinpirole administration

Variables	Before Injection (Mean±SD)	After Injection (Mean±SD)	*t*	*p*
24h sum	6.266±2.282	6.828±2.964	-1.968	0.064
Light phase sum	4.462±1.854	3.245±1.680	4.692	0.001
Dark phase sum	8.070±2.820	10.407±4.556	-2.947	0.016
Mean period	5.618±0.459	4.523±0.657	2.399	0.040
Variation of period	2.367±0.492	2.395±.0.171	-0.168	0.871

Paired t-tests were used for comparison of mean period and standard deviations.

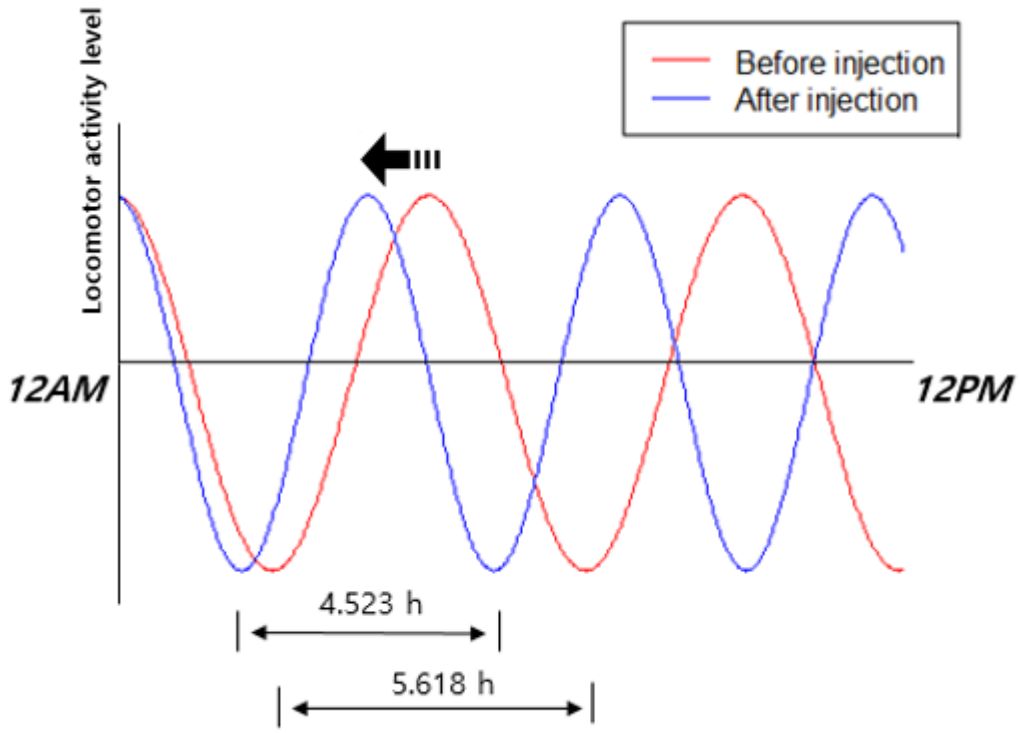

Figure 1. Schematic drawing of ultradian rest-activity rhythms induced by quinpirole injection. A schematic drawing presented the effect of quinpirole administration on the ultradian period of tested mice. On average, quinpirole administration reduced the period of the tested animals from 5.168 hours to 4.523 hours, assuming a constant amplitude.

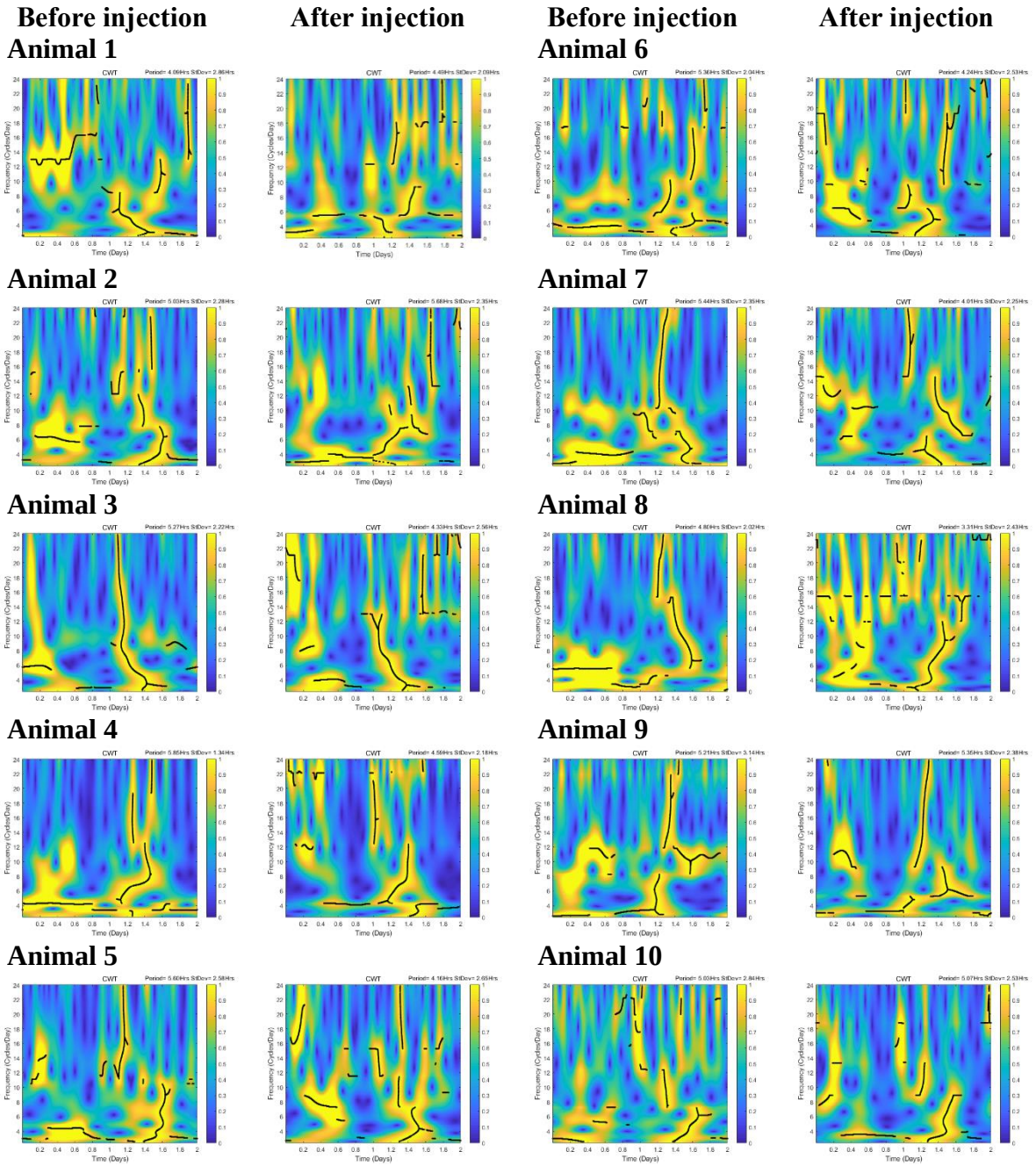

Figure 2. Graphical presentations of ultradian rest-activity rhythms. Each animal exhibited distinct patterns of ultradian rhythms when depicted on heatmaps. The period before quinpirole administration ranged from 4.090 to 5.850 hours, and after the injections, it ranged from 3.310 to 5.680 hours. The variations in period before and after the injections were 1.340 to 3.140 and 2.090 to 2.650, respectively. Dominant ultradian rhythms are indicated by black lines on the heatmaps. CWT: Continuous Wavelet Transform.