

Video Article

Measuring Circadian and Acute Light Responses in Mice using Wheel Running Activity

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Abstract

Circadian rhythms are physiological functions that cycle over a period of approximately 24 hours (circadian- circa: approximate and diem: day)¹. They are responsible for timing our sleep/wake cycles and hormone secretion. Since this timing is not precisely 24-hours, it is synchronized to the solar day by light input. This is accomplished via photic input from the retina to the suprachiasmatic nucleus (SCN) which serves as the master pacemaker synchronizing peripheral clocks in other regions of the brain and peripheral tissues to the environmental light dark cycle³⁻⁷. The alignment of rhythms to this environmental light dark cycle organizes particular physiological events to the correct temporal niche, which is crucial for survival⁸. For example, mice sleep during the day and are active at night. This ability to consolidate activity to either the light or dark portion of the day is referred to as circadian photoentrainment and requires light input to the circadian clock⁹. Activity of mice at night is robust particularly in the presence of a running wheel. Measuring this behavior is a minimally invasive method that can be used to evaluate the functionality of the circadian system as well as light input to this system. Methods that will be covered here are used to examine the circadian clock, light input to this system, as well as the direct influence of light on wheel running behavior.

Video Link

The video component of this article can be found at <https://www.jove.com/video/2463/>

Protocol

1. Equipment Setup

1. Preparation of animal groups is very important when setting up any behavioral experiment. For wheel running activity, all mice need to be male, age matched, and if possible from sibling matings. Ideally younger mice, approximately 3 months old, are used for wheel running activity.
2. Prior to placing any animals on wheels, the room needs to be completely setup. This includes preparing each cage with 1 wheel, 1 wheel revolution probe, a small amount of mouse bedding in the bottom of each cage and ample food and water for 2 weeks.
3. Room lighting is highly important, when setting up wheel running cages it is important to measure the light intensity at each cage to verify that you are getting even illumination in each cage. Furthermore, when choosing a light source, look for one with a broad spectrum covering 450nm- 650nm. Many companies offer "day light" or "full spectrum" fluorescent bulbs, which meet this criterion. (See Figure 1)

2. Experiment Setup

1. When introducing mice to the wheel running cage, do so in a light cycle which closely matches the light/ dark cycle they were previously housed under.
2. Try to evenly distribute mice to better ensure similar environments for all groups. For instance, refrain from clustering all of one group of mice in a particular area such as a single shelf. This could inadvertently introduce an additional variable.
3. Before making any light cycle perturbations or measuring activity, you should give mice approximately one week to acclimate to the new cage, and wheel. Any mice that are not running on the wheels after one week should be removed from the cage and replaced with another mouse.
4. Once you have a cohort of animals stably running on wheels, you can start setting up light/ dark cycles to test different hypotheses. The various light cycles are discussed in detail in part 3.

3. Experimental Assays

1. To assay photoentrainment, start by housing mice in a 12 h light: 12 h dark cycle. An example of this would be lights on from 7:00am-7:00pm while the lights remain off from 7:00pm- 7:00am. Additionally, lowering the light intensity for the 12 h light portion of the cycle will expose any

differences in light sensitivity for photoentrainment. Mice should be given two weeks to photoentrain and before changing light intensities. (Figure 2)

2. An additional aspect of photoentrainment is the ability to re-adjust or re-entrain circadian rhythms to a new light/dark cycle. This can be examined by advancing the light/ dark cycle that mice are housed in such that the lights will turn on and off earlier. Commonly, light cycles are advanced by 6 hours; for instance, instead of a light cycle of 7:00am-7:00pm, it would be 1:00am-1:00pm. This can also be examined by delaying the light cycle. Mice are typically able to re-entrain in 5-7 days. (Figure 3)
3. These paradigms do not, however, fully assess the functionality of the endogenous circadian clock. Housing mice in constant dark conditions can be used to measure the endogenous circadian period also known as the free running period. In nocturnal animals such as wild type mice, the endogenous period is less than 24 hours meaning that they begin running on the wheel a slightly earlier each day. (Figure 2) The exact time of this period varies slightly among different mouse strains. For example, the free running period of C57 mice is approximately 23.3 hours while in BALB/cJ mice it is 22.5 hours¹⁰.
4. After determining the free running period in constant dark conditions light input to the circadian system can be examined through phase shifting. Phase shifting is the ability of an acute light pulse to shift the circadian clock. Phase shifting can be accomplished by administering a short light pulse (15 minutes; 1000lux) during the active phase of mice housed in constant darkness and measuring the onset of activity on the days following this light pulse. (Figure 4) Previous studies have produced response curves (termed phase response curves) that describe the amount and direction of shift based on what time the light is given^{11, 12}. For example, a 15 minute, 1000 lux light pulse given 4 hours after the start of activity (CT16) will lead to a 2 hour delay in activity, while the same light pulse given 8 hours after the start of activity (CT20) will lead to a 2 hour advance. (Figure 4)
5. To test the direct effect of light on wheel running activity, a light pulse can be administered to mice during the dark/active phase. The presence of light at this time will inhibit wheel-running activity. While the length of this light pulse can vary, we have found that a 3-hour pulse starting 2 hours after the start of the dark phase is most informative. (Figure 5) In some cases, it can be useful to consolidate activity prior to this test, you can easily do this by changing this light cycle to a 16 h light: 8 h dark cycle one week prior to administration of the light pulse.
6. This inhibition of wheel running activity directly by light can be examined at all phases of the circadian cycle by using an ultradian (T7) light cycle¹³. This cycle consists of 3.5h light: 3.5 h dark for the duration of 1 week¹³. Mice are not able to photoentrain allowing light pulses to fall at all points in the circadian cycle over the course of this 1-week treatment. (Figure 6)
7. Similar to constant dark conditions, housing mice in constant light will reveal a characteristic free running period. However, this period will be longer than the period found in constant darkness. The length of the free running period in constant light is positively correlated with light intensity¹⁴; as the amount of light increases so does the free running period length. (Figure 7) However, mice rarely show periods greater than 26 hours. Instead, under bright light, their activity will become arrhythmic. For this reason, it is light intensity is an important consideration if you plan to compare period length in constant light.
8. When deciding which order to perform these light perturbations, remember that prior light cycles can affect circadian rhythms. For this reason, it is best to start with the mildest treatments first and progressively move to more severe light treatments. For instance, photoentrainment assays, constant dark, and phase shifts are all very mild and do not seem to lead to long lasting effects. On the other hand, the T7 cycle and constant light are very harsh light treatments and can lead to arrhythmia and should be reserved for the end of the experiment.

4. Analyzing the Data

1. Wheel- running activity is collected and the number of wheel revolutions occurring within a given time period binned. This bin or sampling interval is commonly 10-minutes. Data can be displayed in an excel spreadsheet showing the number of wheel revolutions within each bin. This can be useful for quantifying and comparing wheel revolutions. The data can also be displayed as an actogram whereby the amount of activity in each bin is represented by the height of a black bar like a histogram. (Figure 2)
2. Activity onset is defined as the start of the predominate activity bout. The exact parameters may vary between each individual, but generally, we look for the first bout of activity that lasts for longer than 30 minutes.
3. To analyze the ability of mice to photoentrain, there are several measures that can be looked at. A common method used to consider a mouse to be photoentrained is the observation of a stable relationship between the onset of the mouse's activity and the offset of light as well as a large percentage of the mouse's activity being confined to the dark phase.
4. The onset of activity provides the most information when looking at re-entrainment to an advance of the light/dark cycle, while activity offset is most informative when examining re-entrainment to delays in the light/dark cycle. Mice typically take 5-7 days to completely re-entrain their wheel running activity to a 6-hour advance or delay of the light/dark cycle. To consider a mouse re-entrained, particularly in response to an advance, you should observe a stable relationship between the onset of activity and the offset of light. This relationship should be similar to what was observed prior to the light cycle advance and should persist for at least three days. (Figure 3)
5. To calculate period in both constant dark and constant light, the onsets of activity need to be determined for 2 weeks and fitted with a regression line. As mentioned previously, in wild type mice, the period in constant dark will be less than 24 hours while the period in constant light will be greater than 24 hours. (Figures 2 and 7)
6. When calculating the shift following a light pulse, begin by fitting regression lines to activity onsets for at least 5 days before and 5 days after the light pulse. Calculate the difference between these two regression lines to determine the magnitude of the phase shift. (Figure 4)

5. Representative Results

When looking at wheel running records, you should expect to see several hours of continuous running in each mouse. Because mice are nocturnal, this running, or active phase, will correlate with the dark period in the presence of a light/dark cycle. Mice free running in constant conditions will also have this confined period of sustained activity representing the active phase of their free-running rhythm. In constant darkness, this will be shorter than 24 hours and in constant light, greater than 24 hours. Administration of a 15-minute 1000lux light pulse 4 hours after the onset of activity (CT16) will result in delayed activity onset on the days following this light pulse.

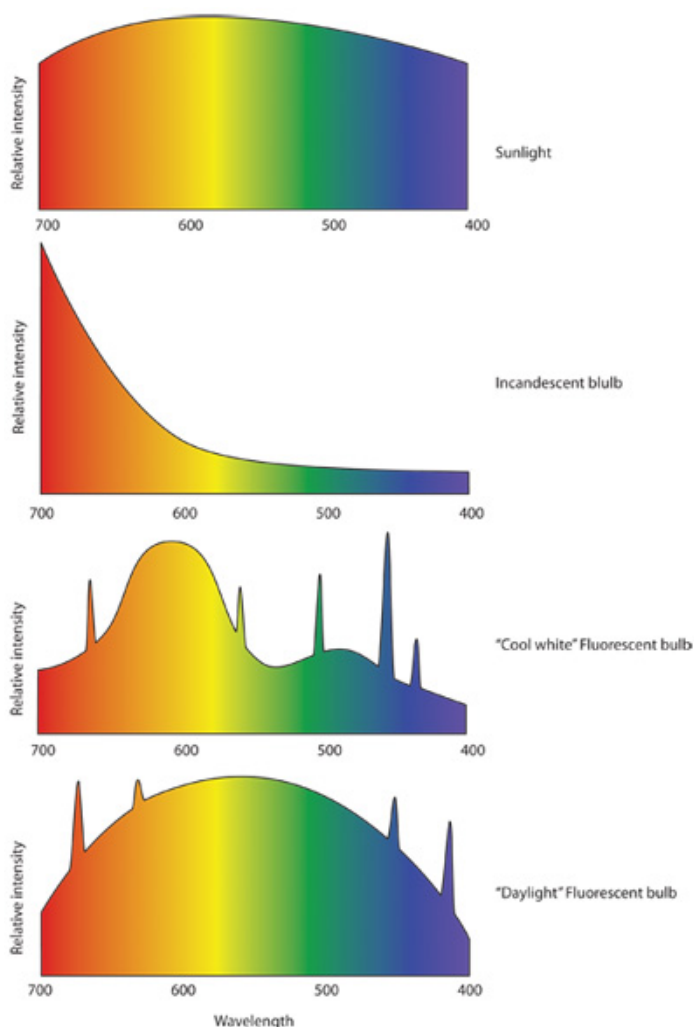


Figure 1: Representative light spectra. A. The light spectrum produced by the sun is broad and relatively unbiased between 400nm and 700nm, which is the visual portion of the light spectrum. B. The spectrum of an incandescent light peaks in the infrared range, which leads to its red bias, with low intensity in the blue range. C. The typical fluorescent bulb, consists of discrete peaks of light which will appear "white" to the eye. D. However, daylight fluorescent bulbs have a higher broad spectrum and discrete frequency peaks. The result is a light source that is not as hot as an incandescent bulb but has a broader spectrum than an average fluorescent bulb.

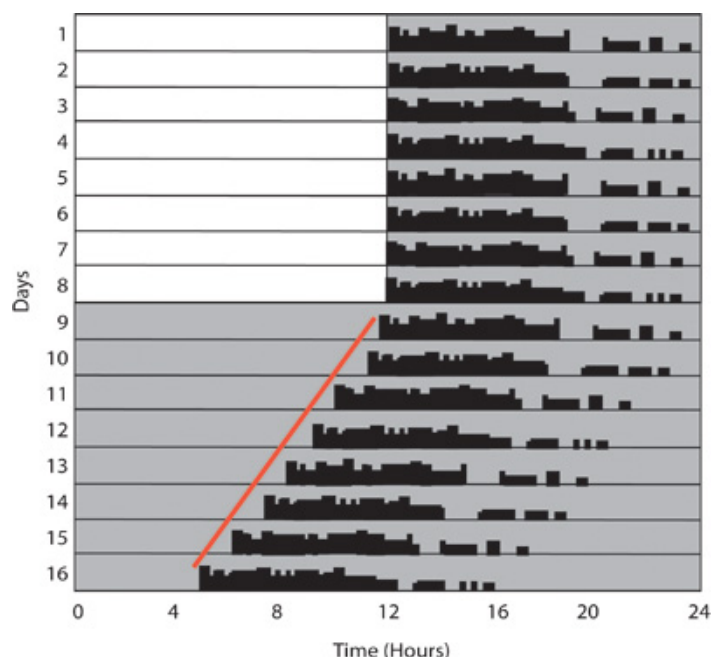


Figure 2: Circadian photoentrainment. The actogram is a graph of activity from a single mouse where consecutive days are plotted on the y-axis, and time is plotted on the x-axis. The black bars represent the number of wheel revolutions, and the background is shaded to give an indication of the light/dark cycle. On days 1-8, the mouse is housed in a 12:12 light: dark cycle. During this time, the mouse starts running at the offset of lights and consolidates its activity to the dark phase. During the light phase, activity is minimal. On day 9, the lights are turned off, leaving the mouse in constant darkness. Without a light cycle, there is no input to the circadian clock, which results in the clock running on its endogenous time. As you can see, activity starts earlier each day an indication that the endogenous clock of this animal is less than 24 hours.

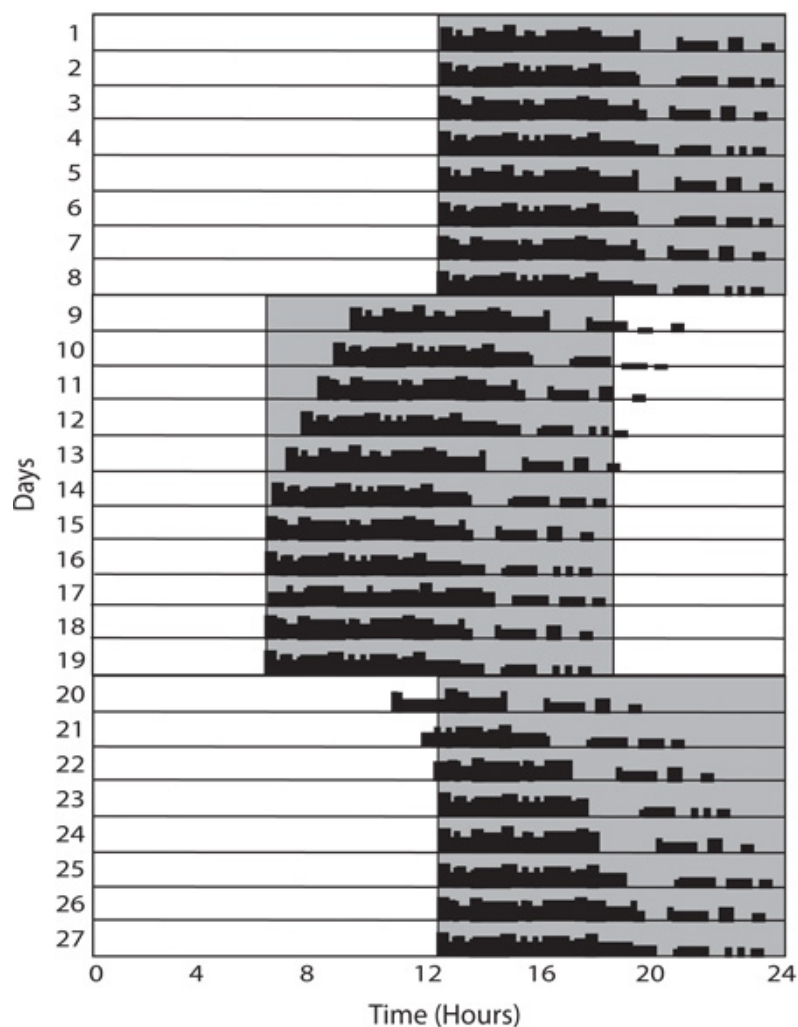


Figure 3: Re-entrainment. Re-entrainment paradigms involve shifting the light cycle either forward or back forcing the circadian clock to adjust to the new light cycle. In this example, the cycle is advanced 6 hours on Day 9 and then delayed 6 hours on Day 20. In both circumstances, several days are required for full re-entrainment, however depending on the direction of movement the effect is more pronounced in either the onset or offset time.

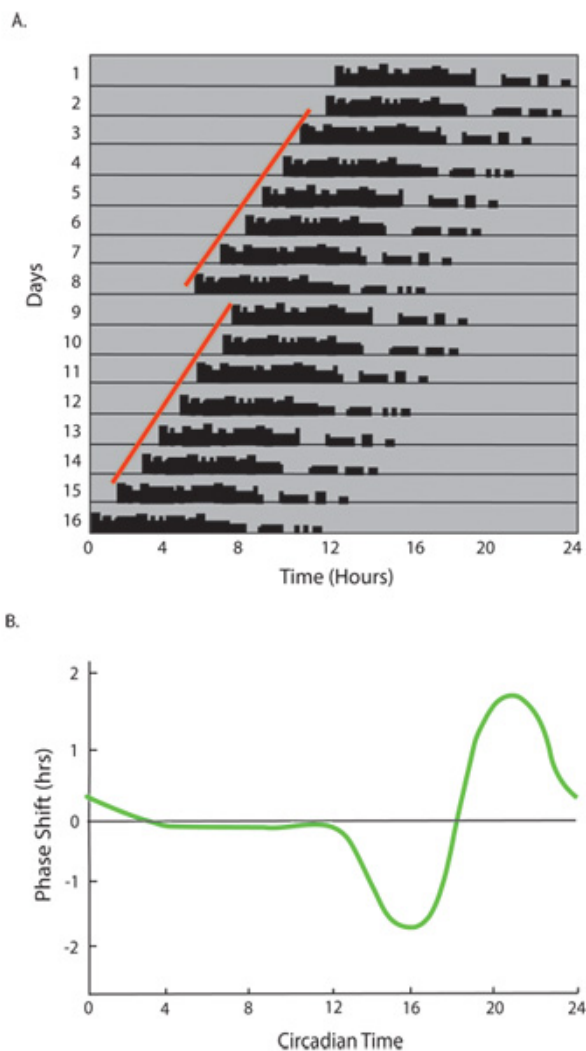


Figure 4: Circadian rhythms shift in response to short pulses of light. A. Animals housed in constant darkness free-run with a period shorter than 24 hours. On Day 8, a 15-minute 1000-lux light pulse is given 4 hours after the onset of activity (CT16). On the following days, a shift in activity onset time is observed. The phase shift is the difference in the animal's predicted onset time (determined from their activity prior to the light pulse) and the time that they actually start running on the days following the light pulse.

B. The phase response curve for mice shows the amount and direction of shift that will occur in response to a brief light pulse at specific circadian times. A light pulse during the subjective day (CT0-12) cannot induce a significant shift, referred to as the dead zone. From CT12-19, a phase delay can be induced with the peak delay occurring in response to a light pulse at CT16. Between CT 19 and 24, the activity rhythm will advance in response to a light pulse with the peak advance occurring in response to a light pulse at CT20.

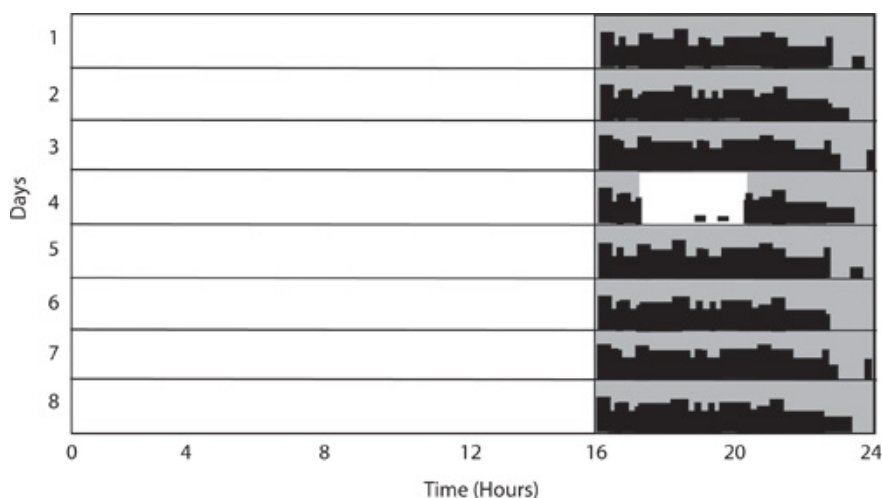


Figure 5: Wheel-running activity is inhibited or masked in response to a light pulse at night. In this example, the light cycle is 16h light: 8h dark and consolidates activity. A 3-hour light pulse begins 2 hours after the lights go off on Day 4. During this time, there is little to no wheel-running activity showing the ability of light to directly inhibit activity. Activity resumes subsequent to the light pulse.

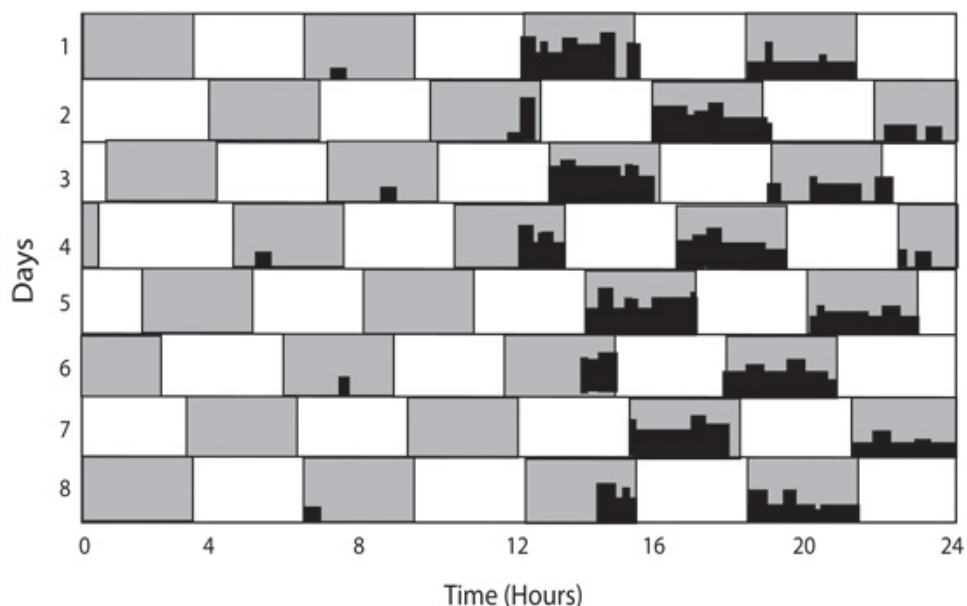


Figure 6: T7 light cycle is used to expose mice to light pulses throughout the circadian cycle. The T7 cycle consists of 3.5 hours of light followed by 3.5 hours of dark. In this cycle, mice will maintain a circadian rhythm, however they will also display masking of wheel running activity during the light pulses. The amount of activity during the light can be compared to the amount of activity during the dark for a measure of masking which is not biased by the circadian time.

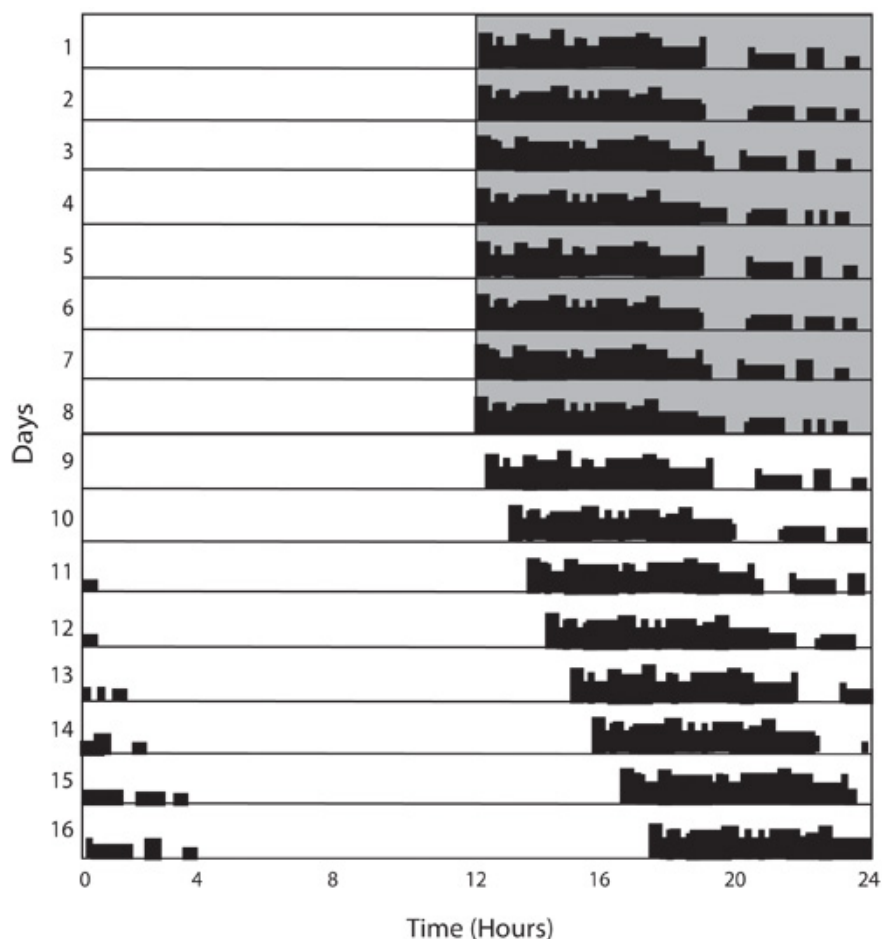


Figure 7: Period lengthening in constant light. Days 1-8 show photoentrainment of the mouse similar to Figure 2, however on Day 9 the mouse is placed in constant light. In response to this condition, mice free-run with a period longer than 24 hours as indicated by the activity onset occurring later each day.

Discussion

Circadian rhythms have been measured and recorded in various organisms throughout history. While we have specifically described the method for recording activity rhythms in mice, this technique can be easily modified to measure rhythms in other rodents such as hamsters and rats, which are often used in circadian studies. However, the freerunning period and time to re-entrain in other organisms will vary. For instance, the freerunning period of a hamster in constant darkness is 24.0 hours, while in mice it is less than 24 hours. In the circumstance, that you are working in an organism, which is not suitable for wheel running activity, many of the same light paradigms can be used, however a different physiological rhythm should be looked at. The most similar measure is general activity. In humans, monkeys, and dogs, researchers often use actigraphy, which measures the amount of movement of the individual. In humans, body temperature and melatonin secretion are also commonly measured and have both been shown to have a circadian rhythm, which is capable of phase shifting and able to re-entrain^{15, 16}.

While we think of these physiological rhythms as discrete occurrences, they are quite interconnected. The activity rhythm of an animal can be closely overlaid with the body temperature rhythm and is the inverse of a sleep rhythm. Furthermore, the melatonin secretion that is often used as a marker of circadian phase, closely resembles the normal sleep rhythm in humans. Because these cycles are so interwoven, the study of one can inform and predict results of others. For instance, *NPAS2* mutant mice, which showed continuous wheel running activity though out the dark portion of a light dark cycle, were later examined for sleep defects¹⁷. These mice were found to sleep less overall, with the decrease occurring during the dark phase, the normal "nap time".

Wheel running activity provides a relatively non-invasive way to examine circadian rhythms. The paradigms described above can be used to complete a detailed analysis of circadian behavior. Additionally, paradigms such as photoentrainment and housing in constant darkness can be used to as an initial survey of circadian phenotypes prior to more concentrated research such as sleep analysis.

Disclosures

No conflicts of interest declared.

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