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Phenotyping Circadian Rhythms in Mice

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Abstract

Circadian rhythms take place with a periodicity of twenty-four hours, temporally following the rotation of the earth around its axis. Examples of circadian rhythms are the sleep/wake cycle, feeding, and hormone secretion. Light powerfully entrains the mammalian clock and assists in keeping animals synchronized to the 24-hour cycle of the earth by activating specific neurons in the “central pacemaker” of the brain, the suprachiasmatic nucleus. Absolute periodicity of an animal can deviate slightly from 24 hours as manifest when an animal is placed into constant dark- or “free running”- conditions. Simple measurements of an organism's activity in free running conditions reveal its intrinsic circadian period. Mice are a particularly useful model for studying circadian rhythmicity due to the ease of genetic manipulation, thus identifying molecular contributors to rhythmicity. Furthermore, their small size allows for monitoring locomotion or activity in their home cage environment with relative ease. Several tasks commonly used to analyze circadian periodicity and plasticity in mice are outlined here including the process of entrainment, determination of tau (period length) in free running conditions, determination of circadian periodicity in response to light disruption (i.e. jet lag studies), and evaluation of clock plasticity in non-twenty-four hour conditions (T-cycles). Studying the properties of circadian periods such as their phase, amplitude, and length in response to photic perturbation, can be particularly useful in understanding how humans respond to jet lag, night shifts, rotating shifts, or other transient or chronic disruption of one's environmental surroundings.

Keywords

circadian; photic entrainment; tau; period; locomotion; phase; amplitude; light

Introduction

Light is an extremely powerful entrainer (or *zeitgeber*, time giver) for the mammalian brain. Light controls circadian rhythmicity in the suprachiasmatic nucleus (SCN) of the hypothalamus by activating melanopsin-containing neurons connecting the retina to the SCN (Gooley et al., 2003). Typically, light functions as an entrainment signal, keeping organisms tethered to the 24-hour light/dark cycle of the Earth. Manipulation of the light phase in rodents provides the opportunity to study mechanisms underlying photic

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entrainment. It assists in understanding what molecules in our central (SCN) and peripheral clocks help us deal with jet lag, night shifts, or other sources of environmental perturbation. The process of photic entrainment (the use of light cycles to influence rhythmicity) will be discussed here.

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Photic entrainment refers to the process by which light (an external *zeitgeber*) provides timing for our endogenous clock. It assists in the process by which our internal period (τ , or τ) is manipulated to match that of our environment. Because most organisms have slightly shorter or slightly longer intrinsic periods than 24-hours, light assists in entraining the internal clock to that of exactly 24-hours. Time is measured in *zeitgeber* (or ZT) hours, with ZT0 being the “lights on” onset and ZT12 being the onset of “lights off” on a 12-hour light, 12-hour dark schedule. Following entrainment to a standard light/dark cycle, mice can be placed in constant darkness, where the true endogenous circadian period can be revealed. In free running conditions, time is then measured in circadian time (or CT), since it is now subject to the endogenous rhythm of the organism, which may be slightly shorter or longer than 24-hours. The same is true for organisms other than mice. Mice generally (but not always) have a period length of slightly less than 24 hours in free running conditions (Figure 1). Figure 1 shows double plotted actograms generated by two different software programs for mice entrained in 12L:12D and then placed under constant dark conditions. Note that the period length of activity is slightly shorter for each of these animals under constant dark conditions and the activity shifts slightly to the left over time. When CT is being measured, the τ of the animal is divided by 24. Thus 1 circadian hour = $\tau/24$. This measurement should be made after 2 weeks in constant dark conditions, preferentially, as the first couple of days in constant dark conditions, the central oscillator will be in transition and true circadian phase will be unable to be measured.

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The pacemaker can be entrained by *zeitgebers* that are continuous (whereby the clock has to speed up or slow down to match the imposed external cycle), or it can be entrained by *zeitgebers* administered transiently, where a phase-shift (ϕ) in the oscillator is produced depending on the time of *zeitgeber* administration. Sometimes, an imposed external period is too short or too long for the endogenous clock to adapt to. In this scenario, the animal is unable to entrain because the imposing cycle has exceeded the range of entrainment for the circadian system of the animal. The time difference in hours between the external, entraining rhythm and that of the organism (as measured by activity onset, body temperature, etc.) is referred to as the phase angle difference (ψ). If entrainment takes place, the phase angle difference between the internal and external rhythms is stable.

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Frequently, light pulses during the night or the subjective night can be used to study the mechanisms underlying resetting of the circadian clock. Depending when the light pulse is administered, activity onsets can either be phase advanced or phase delayed. The direction of this shift depends directly on the time point at which the light is administered. Following a light pulse, a phase response curve (PRCs) can be generated. A PRC plots the phase shift of an animal's activity (generally in hours) vs. the circadian time (again, hours) of the light pulse. In a PRC, a positive shift reflects a phase-advance while a negative shift on the curve reflects a phase delay. (For additional information regarding many aspects of entrainment across a variety of organisms, see *Circadian Systems: Entrainment* by Colin Pittendrigh).

Several hardware setups can be employed to measure behavioral rhythms. Two of the most common and cost effective ways of measuring rhythmicity are via wheel runners, which are placed inside the home cage of the mouse, or infrared sensors, which are typically placed above an animal's home cage. There are distinct advantages to both of these approaches. For example, wheel runners have a greater signal to noise ratio as very minimal activity will be detected from a nocturnal mouse during the light hours. While slightly more rest phase activity will be detected from infrared sensors placed on top of the cage, this “noise” is also informative as it aids in understanding the general activity levels of animals during the rest phase. While the mice may not run much on their wheels at night, they may arouse and groom, eat, etc. This protocol focuses on rhythm detection via infrared sensors but the same analysis can be applied to activity plots generated by either method. In addition to the options for assessing circadian locomotion, animal housing can also be flexible. Some labs have developed custom-made isolation cages, others use isolation cabinets (where multiple cages can be stored) (Figure 2A). These options have great benefits in that temperature, light, and noise can be exquisitely controlled within the cabinet or within an individual cage itself. Other labs studying rodent rhythmicity use rooms that are separated by a door from exterior rooms or halls. In this case, a revolving dark room door is extremely helpful (see Figure 2B), as it limits the possibility of exposure of the animals to the photic cues of the neighboring area. In this case, the light and temperature of the whole room must be controlled and less manipulation for specific animals is possible compared to individually-controlled cages, for example. The great advantage of isolation cabinets and cages is the ability to manipulate conditions for a single animal or small group of animals, while a great advantage of housing animals in a room is that the funds necessary to cage the animals are considerably reduced. The following protocol is geared towards animals housed in a light-protected room.

Note: All protocols using liver animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) or must conform to governmental regulations regarding the care and use of laboratory animals.

Basic Protocol 1

Circadian Phenotyping in Mice- Entrainment and Determination of Period Length (Tau, τ) in Free-Running Conditions

Introductory paragraph—The following protocol steps the researcher through basic procedures and equipment necessary for assessing the endogenous circadian period of a mouse. Generally, most laboratory mice are nocturnal and thus, one should expect to observe the vast majority of activity taking place during the dark phase of a 12-hour light/dark cycle, while the light phase will be mainly void of activity (Figure 3). The inverse would be expected for a diurnal organism. The same is true of circadian time. The activity levels of a nocturnal animal during the subjective day reflected in an actogram generally reveal minimal activity counts compared to the subjective night. For locomotion analysis, animals must be individually housed so the circadian properties associated with locomotion patterns can be attributed to a single animal. While locomotion can be monitored in novel environments, the protocol for measuring circadian rhythms reported here is for a mouse in

its home cage. Novel environments, which typically induce exploration, can mask the response to photic cues and should be avoided. Several hardware components can be placed in or above the cage for locomotion monitoring. Wheel runners or infrared sensors are cost effective ways of easily measuring mouse movement in a time series. Wheel running is typically reported as revolutions over time. Wheel runners sized for mice and infrared motion detectors can be purchased from a number of companies including Starr Life Sciences Corp. Figure 4 demonstrates a motion detector system, by which overall circadian locomotion can be detected and plotted. Using these sensors, the placement of the animal, its food, and its water are as normal, and the sensor is mounted securely to the cage top. In addition to the cost-effectiveness of the infrared system, one benefit is that there is limited disruption to the animal should there be a mechanical issue to deal with, as everything required for circadian locomotion acquisition is located outside of the animal cage. (Note: here, the sensor is shown on cages used in a typical animal facility. See Materials for cage ordering options. Also, the infrared system eliminates the possible behavioral bias that mice may have for running wheels where they need to be “willing” to get on. If using facility cages, the system described below- be it involving wheel runners or infrared sensors- can be built for under \$25,000 to monitor 24 animals at a time. Systems can be scaled up or down depending on the needs of the researcher.) Figure 5 illustrates the flow of information from the cage to the computer using the itemized parts listed in the materials and methods.

Materials

Mice (control and experimental, a minimum of 6 per condition)

Standard Mouse Cages* with bedding, food, water, and nestlets

Cage Body- 365×207×140, polycarbonate (Starr Life Sciences Corp 1284L001)
(OPTIONAL)

Wire Lid- steel inner lid with hinged divider (Starr Life Sciences Corp 1284L116)
(OPTIONAL)

Enclosed Room or light protected cages (insulation cabinets)

Infrared Cage Top Motion Detector**, (Starr Life Sciences Corp 130-0065-AA, or BIO-LYNX in Canada, 130-0065-AA)

QA4 Activity Input Modules (One module integrates 4 motion detectors or wheel runners)
(Starr Life Sciences Corp 130-0050-00)

DP-24 Data Port (Starr Life Sciences Corp 840-0024-00, or BIO-LYNX 840-0024-00).
One DP24 integrates 24 sensors, 6 QA4 modules

C-50 Cable, 10m (Starr Life Sciences Corp, 1076589 or BIO-LYNX 060-0045-10)

VitalView Software with PCI Card (Starr Life Sciences Corp. 1098589 or BIO-LYNX 855-0035-00) PC-based data acquisition and analysis

ClockLabs (Coulbourn Instruments, ACT-500), (required for the generation of single and double plotted actograms, etc. from VitalView acquired data. Requires MatLabs.)

Light Timer (can be purchased from home improvement stores or may already be installed in animal facility)

Lux Meter (can be purchased from home improvement stores)

Night Vision Goggles with infrared beam (can be acquired from a variety of stores). Alternatively, dim red lighting can be used- see protocol.

Computer Windows required for Vital View Software

Opaque Tape and/or Black Plastic Sheeting to cover all light sources in the animal room (such as power lights on computers, activity monitors, etc.)

*- refers to cages that are not light-protected but rather housed in an enclosed room

****- or Running Wheel**, 4.5 diameter with reed switch (Starr Life Sciences Corp 610-0003-00). Wheel runners suitable for mice are also available at Columbus Instruments (www.colinst.com), product number 0297-0521.

Protocol steps

1. Place animal cages in a room that is protected from external light cues. Alternatively, light-protected cages can be used. Each cage should have similar enrichment or lack thereof.
2. Using a light timer, set the light schedule on a 12-hour light/12-hour dark cycle. Alternatively, 14-hour light/10 hour dark cycles are commonly used in rodent studies. *Lights on in the chamber/room is considered zeitgeber time 0 (i.e. ZT0) and lights-off in the room is considered ZT12 when the lights are set on a 12-hour light/12-hour dark schedule.*
3. Adjust main light sources such that approximately 100-300 lux of light is equally distributed to all animals. For example, if animals are housed on shelving, attempt to avoid double stacking or placing the animals in a manner that results in some animals receiving significantly less light than others.
4. Conceal all possible light sources that may transmit additional visual light to the animal during the dark phase. If present in the entrainment room, light emanating from computer screens, hardware power buttons, etc. should be covered with an opaque, light impenetrable material.
5. Habituate animals in the protected room or light-protected circadian chambers for 10-14 days. *This is considered an acceptable length of time for adequate entrainment of internal rhythms to the entrainment cycle. While eating, drinking, and locomotion typically align within a few days, internal rhythms generally take longer to fully entrain.*

6. Release the animals into constant dark conditions by resetting the timer to stay off after the dark cycle and continue measurements for the desired length of time in constant darkness. Note: the oscillator will go through several cycles prior to reaching stable entrainment. Thus, several days of transient behavior will be noted until the true circadian phase can be measured.) Following the transient period, locomotion should be monitored for a minimum of two weeks for more accurate τ measurements.
7. Files can be generated using Vital View. Within Vital View, individual channels are separated and files are created for each animal. The data within these files can be opened and analyzed using Excel among other similar programs. In addition, these files can be transferred to other programs (such as ActiView or ClockLabs) to produce periodograms and actograms. (*Note: each of these programs comes with thorough manuals regarding software use and options.*)
8. Calculation of Circadian Time (CT) is performed by determination of the daily onset of activity (always referred to as CT12) in reference to a 24-hour day. Daily activity onsets should be measured for at least six days (but preferably longer) to determine internal τ . Thus, at the end of stable entrainment to constant conditions, one must calculate the difference in real time of CT12 on day 2 vs CT12 on day 1. If CT12 on day 2 arrives earlier (i.e. activity onset occurs earlier than the previous day), the τ will be less than 24 hours. One circadian hour will be measured accordingly: 1 circadian hour = $\tau - 24$.
9. Actograms plotted from individual activity files can be created using ActiView or ClockLabs. *Generally, actograms are double-plotted meaning that two days are plotted on each line, showing the second of the two days on the right. This is helpful in visualizing non-24 hour rhythms when present.*

Alternate Protocol 1

Determination of the Range of Entrainment Using T-Cycles

Introductory paragraph—The circadian response to non-24 hour cycles (or T cycles) is commonly studied to understand the role of the circadian clock in photoperiodic time measurement. T-cycles are periods that are shorter or longer than 24-hours, where $L+D=T$. Often, T cycles are used to study the phase angle of entrainment as well as understanding limitations to entrainment. T-cycles are often administered as short photoperiods, but short photoperiodism is not necessarily a requirement of a T-cycle. For example, the LD cycle could be 11L:12D ($T=23$), 7L:12D ($T=19$), or 11L:11D ($T=22$). All of these are T-cycles that are less than 24-hours. Alternatively, the LD cycle could be longer than 24 hours. For example, the LD cycle could be 6L:20D ($T=26$) or 7L:22D ($T=29$), both of which would be attempting to entrain an animal in a period longer than its normal τ . Figure 6 shows two actograms where animals were stably entrained to T-cycles of different lengths- one in which $T=22$ and another in which $T=26$. In each situation, animals entrained to the cycle and their subsequent free running behavior reflected the change in T-cycle length. Sometimes, an animal cannot entrain to the T-cycle administered because it has exceeded

the range of entrainment. This would likely be the case for $T=19$ and $T=29$, for example. T -cycles are useful in determining the limits of circadian entrainment.

Materials

The same materials used for protocol 1 can be used for T -cycle administration and locomotion analysis following T -cycles.

Protocol steps

1. Entrain animals in a 12-hour light/12-hour dark cycle.
2. Set the light timer in the cage or the room so as to apply the desired T cycle, where T is $<$ or $>$ 24 hours.
3. Wait until stable entrainment to the T -cycle occurs. (*Note that some T cycles will be outside the limits of entrainment for the central clock. Thus stable entrainment will never occur.*)
4. Place the animals in constant darkness to evaluate the endogenous period following T cycle entrainment, recording for a minimum of 2 weeks.

Support Protocol 1

Mimicking Jet Lag in Rodents

Introductory paragraph—Schedules that disturb the circadian clock, such as travelling across time zones or shift work, produce fatigue and sleep disturbances in addition to a number of other symptoms that can produce substantial irritability. This is known collectively as jet lag and is a result of our internal clocks being temporarily out of sync with the external time. Studies mimicking human jet lag are relatively easy to perform in mice. For example, a delay in the LD cycle models westward flights while an advance in the LD cycle simulates taking an eastward bound flight. In most WT mice a phase delay is typically overcome behaviorally a couple of days faster than a phase advance imposed on the animal.

The use of jet lag protocols in mice has been useful in determining the molecular mechanisms underlying reentrainment. For example, recent work looking at vasopressin receptor knockout mice has demonstrated the importance of vasopressin signaling in resistance to jet lag recovery (Yamaguchi et al., 2013). While many jet lag experiments attempt to ascertain the circadian requirements for recovery from acute circadian disruption in the mouse, chronic jet lag experiments can also be used to mimic frequent travel and/or rotating shift work. Such chronic jet lag has been reported to be much more deleterious to animal health and longevity (Davidson et al., 2006).

Materials

The same materials used for the Basic Protocol 1 can be used for jet lag experiments.

Protocol steps

1. Following 14 days of entrainment in a LD cycle (for example, 12-hour light, 12-hour dark), delay or advance the LD cycle by the desired amount. (**Example: 7 hour phase delay** If ZT0 was at 6 am and ZT12 was at 6 pm in the entrainment conditions, change the light cycle such that the light onset now begins at 11 pm and the offset is at 11 am. This will have produced a 7 hour phase-delay of the previous cycle.)
2. Following alterations in the light cycle that produce an advance or delay, constantly record animal behavior for several weeks in free running conditions, ensuring that stable entrainment finally occurs, a process that will take several days at a minimum.
3. Optional: for chronic jet lag experiments, animals may be phase advanced by 8 hours every other day for 10 days and then released into constant darkness. Alternatively, alternating phase advance and phased delays may be used to mimic repeated circadian disruption in opposite directions. Among others, alternate protocols include a weekly phase advance or phase delay perturbation (i.e. once a week) (Davidson et al., 2006).

Commentary

Background Information—Wheel runners and motion detectors remain some of the most common equipment used to phenotype circadian rhythms in mice. While wheel runners show less “noise” during the rest phase, thus making it slightly easier to determine activity onset, motion detectors give an accurate output of total activity during the rest and active phases. Other parameters can be measured during the circadian cycle, such as food and water intake and energy expenditure. Such measurements are often taken in cages designed to measure metabolic inputs and outputs. However, with technology advancing, more opportunities are available for all of these measurements to be taken in one cage, sometimes custom made, and for weeks to months at a time. Nevertheless, for simple circadian phenotyping using running activity or overall movement as a readout, multiple systems are available for measuring large numbers of animals at once at relatively low cost.

Critical Parameters—Several common pitfalls are easily overlooked when performing circadian phenotyping. Firstly, it is very important that mice are disrupted minimally by cage changing, water and food swapping, etc. immediately after light perturbation. Such interference can affect the circadian response to photoperiod manipulation. When possible, wait several days following light manipulation to attend to feeding and bedding needs of the animal. A second common mistake when performing circadian experiments is to unintentionally introduce a phase shift during yearly time changes. When conducting experiments during these critical times, be sure to turn off options for adjusting for daylight savings time on your recording software as well as on the computer's operating system software. These can automatically introduce an unintentional phase shift to the animal.

Because so many non-photoc cues can produce effects that confuse interpretation of circadian locomotion data, a routine diary of animal perturbation should be included in

every experiment. For example, it is useful to know whether a surge of rest-phase activity one day is due to routine cage changing and not some unexpected temperature change or hardware malfunction. In addition to keeping a diary of routine cage and mouse maintenance, the light/dark cycle should be regularly checked. For example, under 12-hour LD entrainment conditions, confirm that ZT0 coincides with the expected “lights on” and that ZT12 coincides with the expected “lights off”. When checking lighting conditions in anticipated constant dark conditions, check the mice using infrared goggles. Alternatively a dim red light can be used as it lacks the ability to reset the central pacemaker.

Troubleshooting—There are three major areas to troubleshoot when assessing circadian locomotion data. While not necessarily common, the three most familiar causes of disruption to appropriate recording include:

1. Unintended photic disruption from a light or sensor not working or not set properly.
2. Malfunctioning hardware connecting individual sensors to the main data port or alternatively, from the main data port to the computer.
3. Impaired animal health and well being

Each of these three areas should be addressed when data acquisition is abnormal. If a mouse is sick or aging, activity patterns can be drastically different compared to a healthy animal. In addition, if a light timer is not working, is set incorrectly, or is in control of a bulb or bulbs that are no longer transmitting light, drastic and rapid changes can be observed in an animal's locomotion patterns. Finally, when recording has been consistent but suddenly stops, it is most often due to a problem with data transmission. For example, cords connecting the individual sensors to a QA4 module may be impaired. Sometime, wires transmitting information from the QA4 to the main data port or, alternatively, from the data port to the computer malfunction. This typically produces an immediate loss of activity recording for a particular cage or set of cages. During recording, activity levels should frequently be monitored such that problems can be dealt with quickly.

Specific strains of mice also pose a problem for certain circadian tasks. While most mice are rhythmic in constant dark conditions, some are not. Sometimes a mouse will display abnormal and unstable rhythms in constant dark conditions, which makes experiments such as phase resetting difficult to perform. In this case, alternative protocols exist (Aschoff, 1965), reviewed in (Jud et al., 2005). In addition to complications in free running rhythmicity, there is tremendous variability between strains in terms of period length. Thus, only within-strain comparisons should be made when attempting to attribute a circadian phenotype to a specific gene, for example.

Anticipated Results—With these brief protocols, one can easily assess whether a rodent of interest is rhythmic (usually defined as maintaining rhythmicity in constant dark conditions), whether it responds normally to a light pulse given at a specific time, and finally, whether clock plasticity is limited or, alternatively, expanded so as to adjust to photoperiods of different lengths. All of these phenotypes ultimately reflect the function of the circadian pacemaker in the mouse and its ability to respond to photic cues. Appropriate interpretation depends strictly on using the proper controls. For example, if heterozygous

mating pairs are set up to generate wild-type, heterozygous, and homozygous progeny for a specific gene, the heterozygous and homozygous animals should be compared to their own wild-type control and not some other “wild-type” animal of a different strain or mixed genetic background. Conversely, within the same strain of animals, care should be taken to age-match animals as closely as possible. Figure 7A demonstrates the free-running period of a young (9-month old) mouse compared with an aged (22-month old) mouse. While the young mouse displays a free-running period of approximately 23.5 hours, the aged mouse has a significantly longer period of almost 24-hours (Valentinuzzi et al., 1997). Figure 7B demonstrates the distribution of free-running period lengths in 46 mice of the CD-1 strain (Refinetti, 2001). While the average tau of the group is 23.6 hours, there is substantial variation among individual mice comprising the group. Thus, only within the right comparison and with sufficient N, can much knowledge can be gained from circadian phenotyping.

Time Considerations—Circadian phenotyping is not a short procedure. Firstly, a 1-2 week entrainment period is unavoidable if the investigator wants to limit noise and excess variability from animal to animal. Even stress on an animal during shipping, for example, can alter the circadian properties transiently. Shipping mice long distances often means that animals are not kept in constant light/dark conditions and if they are, it is unlikely that they are in sync with the light/dark cycles of one's own animal housing arrangement. Thus, entrainment of the animals to the desired light/dark cycle is a necessary first step in circadian phenotyping. Secondly, release of an animal into constant darkness for ascertaining period length is also a lengthy procedure. Depending on the nature of the previous photic manipulation, the central clock goes through a transition stage, which must be completed prior to measuring circadian period. Once the transition phase is complete (usually several days), the animal should be recorded for a couple to several weeks, preferably, for more accurate tau measurements. Thus, one could expect a typical circadian phenotyping experiment to take a minimum of one month. Depending on the extent of photomanipulation involved, or the addition of other variables such as feeding restriction, timed feeding, etc. some experiments may take much longer.

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Figure 1. Actogram Plotting

Two actograms (A and B) graphed by two different programs (VitalView and CLOCKLABS, respectively) each showing the activity of a mouse in a 12-hour light/12-hour dark cycle (12L:12D) and then transitioned into constant darkness. Double plotted actograms (where the x axis=48 hours) show two days of activity on one line, with the first day's activity preceding the activity of the second day. Such a plot assists in visualization of variance from the 24-hour cycle in constant dark conditions. Often the actogram is accompanied by additional information such as the time of lights on in the room/cage (i.e. 06:00 in actogram A), the time of lights off, the start date and resolution of activity monitoring, etc. In addition, the period length in constant darkness can be calculated and is shown in actogram B as the "Fit 1 tau"- 23.75 hours).

A- Temperature and light-controlled isolation cabinets (see Phenome Technologies, <http://www.phenometechnologies.com>)

B- Revolving dark room door

Figure 2. Multiple housing options exist for circadian phenotyping

(A) Isolation cabinets of varying shapes and sizes can be used for storing animals during circadian locomotion capture. Such cabinets are temperature and light controlled and therefore provide superior control over several zeitgebers that can affect rhythmicity. Such cabinets can be purchased from several companies including Phenome Technologies. (B) Revolving dark room doors are a useful way to separate animals housed in standard cages and together in a large room. These doors allow entry without risking light contamination from the adjoining area. Dark room revolving doors can typically be installed by the resident carpentry staff at the research institution. Regular entries can be replaced with revolving doors and additional holes can be drilled into the wall for the transfer of wires from the circadian equipment to computers, etc. that are housed in an exterior room.

Figure 3. Double plotted actogram of locomotion measured using an infrared sensor
Locomotion for a laboratory mouse under a 12-hour light/12-hour dark cycle. Double plotted data reveals the relative lack of movement during the light phase compared to the dark phase. (Data generated by ActiView and visualized by VitalView. Other software- such as ClockLabs from Actimetrics- is available for the derivation of actograms as well.) Note the reduced but present activity counts generated during the animal's rest phase (06:00-18:00). These counts are likely a reflection of animal grooming, eating, or shifting within the cage. Less resting phase activity counts are generally detected when measuring locomotion via wheel runners compared to the infrared sensors.

Figure 4. Motion detector setup in a standard cage

Infrared motion detectors mount easily to standard mouse cages via small nuts and bolts that prohibit movement of the sensor. Rodent movement can be reliably detected while still using the typical filter tops used commonly in standard vivariums. Because the detector mounts on top of the cage (A), the animal can be housed with standard bedding and nestlet(s) and no modifications need to be made for alternative food and water placement.

Figure 5. Flow of information from the animal cage to the investigator

Sensors mounted on the top of each cage (A) plug into a QA4 sensor (B), which can integrate up to 4 sensors. Six QA4 modules can simultaneously plug into one Data Port 24 (C), thus accommodating 24 animals at once. The Data Port 24 is connected to a PCI card on a PC via a C-50 cable. Multiple Data Ports can be used simultaneously.

Stable entrainment to T-cycles can persist for months in young animals.
Azzi et al, Nature Neuroscience 2013

Figure 6. Entrainment to T-cycles

Exposure of an animal to non-24 hour cycles (T-cycles) can reveal the limits of entrainment. Different entrainment profiles depend on the length of the T-cycle and the genetic or behavioral perturbation under which a mouse has gone. Stable entrainment to a T-cycle can persist for many weeks (Azzi et al., 2013). This figure displays disparate free-running activity in mice undergoing entrainment to short and long T cycles. Free-running activity is stable for many weeks following T-cycle entrainment.

Figure 7.

Expected Variations in Free Running Tau. The free-running period of mice (generally considered to be approximately 23.6 hours) can vary substantially across and within strains. (A) Variability in period length of a young animal (nine months, top panel) and an aged mouse (22 months, bottom panel) where the period length of the young animal is approximately 23.5 hours, while the older is close to 24 hours (Valentinuzzi et al., 1997). (B) Average period length distribution of 46 mice of the CD-1 strain in constant dark conditions (Refinetti, 2001).