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Public Statement

Animal Care Practices in Experiments on Biological Rhythms and Sleep: Report of the Joint Task Force of the Society for Research on Biological Rhythms and the Sleep Research Society

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Many physiological and molecular processes are strongly rhythmic and profoundly influenced by sleep. The continuing effort of biological, medical, and veterinary science to understand the temporal organization of cellular, physiological, behavioral and cognitive function holds great promise for the improvement of the welfare of animals and human beings. As a result, attending veterinarians and IACUC are often charged with the responsibility of evaluating experiments on such rhythms or the effects of sleep (or its deprivation) in vertebrate animals. To produce interpretable data, animals used in such research must often be maintained in carefully controlled (often constant) conditions with minimal disruption. The lighting environment must be strictly controlled, frequent changes of cages and bedding are undesirable, and daily visual checks are often not possible. Thus deviations from the standard housing procedures specified in the *Guide for the Care and Use of Laboratory Animals* are often necessary. This report reviews requirements for experiments on biological rhythms and sleep and discusses how scientific considerations can be reconciled with the recommendations of the *Guide*.

Abbreviations: SRBR, Society for Research on Biological Rhythms; SRS, Sleep Research Society

Research on biological rhythms and sleep in vertebrates has contributed greatly to the wellbeing of both humans and animals over many decades and will continue to do so for the foreseeable future. Understanding of the temporal organization of cellular, molecular, physiologic, behavioral and cognitive function has clear beneficial applications. For example, biological rhythms and sleep have obvious effects on psychomotor vigilance and susceptibility to accidents.[16,23] In addition, an enormous literature documents the effects of biological rhythms and sleep on basic cellular functions, and their significant and sometimes previously unforeseen influences on growth and development, neurodegeneration and tissue repair, depression, addiction, metabolic disorders, immunity, fertility, and susceptibility to heart failure, cancer, and other diseases.[2,8,10,12,17,35-37,39,41,44,45]

Researchers from many specialized fields are actively engaged in this research, and interdisciplinary approaches have proven highly successful. Many investigators are members of the Society for Research on Biological Rhythms (SRBR) and the Sleep Research Society (SRS). Typically these investigators are also members of other professional societies. They use conventionally accepted methods for care and treatment of laboratory species, wild animals, or both. In common with other biologists, they contribute to scientific knowledge by testing hypotheses through well-controlled experiments. Knowledge about the mechanisms that generate ultradian, circadian, and longer period rhythms as well as the cycling between sleep and waking and the influences that such mechanisms exert on biological processes can be gained through ethical scientific research conducted within the regulatory guidelines that govern animal studies in North America, the European community, and Asia. However, accurate scientific information can only be obtained when experimental designs, apparatus, and environmental conditions crucial to the clear interpretation of the data are used.[28] IACUC and attending veterinarians are increasingly consulted to assess protocols designed to study behavioral processes and physiological and molecular mechanisms that control biological rhythms and sleep. Our intent in this document is to discuss some of the issues relevant to such evaluation.

Principles of and Terms Used in Biological Rhythm Research

To appreciate the specific requirements for research in biological rhythms, it is necessary to understand fundamental principles of biological timing. A biological rhythm is defined as the recurrence of an event at relatively regular intervals. Characterization of a rhythm typically requires observation of

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the system under study over an interval of time sufficient to confirm and quantify the regularity of its period and its phase. Such rhythms cover a vast range of frequencies and organizational complexities. At one extreme are high-frequency neuronal action potential rhythms; at the other are multiyear fluctuations in animal populations. Whereas many members of the SRBR and SRS concentrate their research on circadian rhythms (those whose period approximates 24 h), ultradian and infradian rhythms (those whose periods are less than or greater than 1 d, respectively) are also of biological interest and importance and are also studied. Remarkably, many of these rhythms are internally generated: they arise from genetically determined, but environmentally regulated, processes that persist within organisms and are often observable down to the level of single cells. Similar research questions are often asked about rhythms regardless of frequency. Among the major questions in the field are: (a) What is the mechanism that generates the rhythm? (b) Are the rhythms endogenous, and how is their period and phase affected by environmental cues? (c) How do multiple oscillators within and between cells couple or otherwise influence one another? (d) What are the effector mechanisms that allow the internal clock to rhythmically modulate and coordinate a large number of physiological and behavioral variables? (e) What are the adverse health effects of the disruption of normal rhythmicity? and (f) What neural pathways underlie the regulation of sleep and wakefulness and how does the circadian system interact with sleep–wake regulatory systems?

Entraining Cues and Environmental Influences on Biological Rhythms

Periodic cues from the environment typically entrain internal clocks, that is, coordinate them in such a way that the internal rhythm adopts the same period as the external environment. To understand this critical process, investigators must often maintain animals under conditions quite different from typical animal housing environments. For example, the animals may be housed under different daylengths (for example, short or long photoperiods), particularly when seasonal fluctuations in biological function are of interest. These changes may often be profound but not evident when standard (for example, unchanging 12:12-h light:dark cycle) conditions are imposed. A critical assessment may require maintaining animals under constant conditions (typically constant dim light or complete darkness) to evaluate the persistence and stability of the biological oscillator under nonentrained conditions. In addition, the study of the important process of entrainment may require maintenance under conditions in which the period of the light:dark cycle differs from 24 h (that is, ahemeral conditions). Other studies may require manipulation of the intensity and wavelength of light, presentation of light pulses at specific circadian phases, or maintenance in constant light. Studies of seasonal physiology and behavior may require housing experimental animals in cold (for example, 5 °C) or constant-dark conditions (or both) for months at a time. The imposed conditions typically fall within the animal's natural homeostatic ability to adapt physiologically or behaviorally to the environment, and therefore they are not especially detrimental to the health and welfare of the subjects, yet they may reveal important principles of biological function. For example, short photoperiods typical of winter may terminate reproduction in some seasonal breeders.[7,18] Research has shown convincingly that this outcome does not occur because of a deleterious effect of too much darkness, but it rather is due to a function of the circadian clock that mediates estimation of day length, thereby allowing reproduction to be adaptively phased with regard to season. Such knowledge has had significant practical consequences for the development of successful breeding programs in a variety of species.

Specific environmental conditions must sometimes be imposed for a considerable period of time. For example, reproductive changes associated with photoperiodism are measured over the course of weeks or months, whereas circannual rhythms can be studied only over the course of years. Even evaluation of a circadian rhythm often requires establishing a stable baseline, performing a manipulation, and documenting the effects of the manipulation over a number of subsequent cycles (for example, 30 to 100 d with minimal disturbance of the animal). Each stage of this process can be time-consuming, but these methods allow precision and statistical power because each animal provides its own control data. In alternative designs, larger numbers of subjects may be compared directly with an independent control group to reduce the time devoted to an experiment.

Different species respond to different environmental stimuli, including not only light[33] but also variations in temperature, food, electromagnetic radiation, sound, social interactions, and other cues.[1] Species differ considerably in their responsiveness to external stimuli. The simple act of transferring an animal between cages, or changing the bedding, can induce arousal and thus disrupt circadian rhythms or change their phase.[42] Even a few milliseconds of light at a particular phase of the cycle may be sufficient to grossly alter activity patterns or reproductive capacity. Such considerations require strict control of all intrusive factors. Experimental subjects must be maintained and managed carefully to avoid disruption of the experiment by undesirable and confounding environmental stimuli. In many rhythm studies, cage change schedules of twice or even once each week may not be consistent with valid experimental design and data collection.[30,40] Inappropriate environmental stimuli increase the variability of results, requiring the use of larger numbers of animals than would otherwise be necessary. Thus, overly restrictive animal maintenance requirements may result in an unnecessary increase in the number of animals used.

Circadian Rhythms, Sleep, and Wakefulness

Endogenous circadian oscillators have important influences on the pattern and occurrence of sleep and wakefulness. Investigation of the brain mechanisms that generate sleep–wake cycles and research on the effects of normal and disordered sleep on behavior and physiology share many of the unique animal care and use requirements with research on biological rhythms. For example, measuring stable patterns of sleep and waking requires continuous recording of electrophysiological (electroencephalogram and electromyogram) and physiological variables (for example, body temperature) for extended periods of time. The requirements for control of environmental factors such as light, ambient noise, and ambient temperature are as rigorous as those for studies of other biological rhythms. Typically animals must be isolated from conspecifics during data collection to prevent cagemates from damaging the recording cables that are used in many laboratories for chronic electrophysiological data collection and to reduce variance in sleep–wake amounts that can arise from social interactions.

Studying the effect of inadequate sleep on brain function and physiological variables often requires restriction of an animal's opportunity to sleep. Because sleep is generated intrinsically, sleep deprivation poses special challenges compared with those for simple food and water restriction, requiring some type of stimuli or intervention that prevents animals from falling asleep. Some methods involve placing animals on a small

platform (for example, an inverted flowerpot), which prevents them from adopting postures that are compatible with sleep.[38] Either intermittent or continuous forced locomotion, achieved in various ways (for example, slowly rotating disk, slow moving treadmill), has been used to achieve sleep deprivation over the course of multiple days. The method of choice for shorter term sleep restriction involves 'gentle handling,' in which the experimenter stimulates the animal by various means (tapping on the cage, placing novel objects in the cage, touching the vibrissae or fur with a cotton swab, and so forth) at the initial behavioral or electrophysiologic signs of sleep. Many prevalent clinical sleep disorders (for example, sleep apnea) involve repeated brief interruptions of sleep (sleep fragmentation) rather than total sleep loss. Therefore, many studies attempt to mimic such clinical sleep pathologies by periodically interrupting sleep at intervals of several seconds to minutes.

The nonspecific stress effects caused by the stimuli or interventions used to prevent sleep are a significant concern in all sleep-restriction studies. Such considerations are important for both animal welfare and the integrity of the data, because effects due to loss of sleep must be differentiated from those that are more generally attributable to stress. The nature of the interventions used to prevent sleep and the duration of deprivation are important determinants of associated stress. A few hours of sleep restriction with gentle handling will present different concerns about stress than do multiple days of housing of animals on a small platform over water.[16] Both the investigator who plans the experiment and the IACUC that reviews the protocol must consider the nature and duration of sleep restriction in the context of obtaining a valid answer to a scientific question while balancing effects on animal welfare. The sophisticated nature of collecting data on sleep and circadian rhythms may require specialized equipment and housing chambers not available in many animal facilities. If such equipment cannot be accommodated in the regular animal care facilities, prolonged housing in an approved location outside of the facilities may be necessary to successfully complete the experiment.

Compliance of Biological Rhythms Studies with Regulations

The SRBR and the SRS accept without reservation that animals used in research must be accorded the highest standards of veterinary care consistent with the conduct of the research. Guidelines must conform to *U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (IRAC 1985), US Department of Agriculture Animal Welfare Regulations, Public Health Service policy (see: http://grants.nih.gov/grants/olaw/references/phspol.htm), and the Animal Welfare Act of 1990 (Public Law 89-544). Investigators must comply with the specifications including those for environment, housing, and management of vertebrate animals outlined in the *Guide for the Care and Use of Laboratory Animals*[15] (eighth edition; referred to hereafter as the *Guide*), with modifications as necessary for the successful conduct of the experiments. No portion of the *Guide*, or the public law with which it is designed to facilitate compliance, is intended to interfere with conduct of scientifically valuable research. The IACUC holds discretionary power to approve scientifically justified deviations from standard practices of daily visual monitoring and lighting or other environmental conditions, as long as these deviations are necessary for successful completion of interpretable experiments and occur within the range of conditions suitable for the health and welfare of the animal subjects. The *Guide* recognizes that lighting intensities may be modified to accommodate the needs of research protocols as long as the system provides for observation and care of the animals. This goal often can be achieved by remote monitoring, such as computer-assisted detection of activity, temperature, or other relevant measures; direct visual observation of animals is not necessary if a computer record reveals that its activity in a running wheel or other monitoring device is consistent with that of a robust and healthy subject and has not deviated from the normal pattern. This assurance can be accomplished by remote evaluation of records, typically on a daily basis. Furthermore, infrared viewers or other devices can be used to observe animals without disturbing them and thereby compromising the experiment.

Biological rhythm research is highly sensitive to environmental perturbation, and administrators and veterinary staff should become familiar with the specific requirements that are necessary for valid studies of biological rhythms and sleep. Cooperation between investigators, animal care staff, IACUC members and attending veterinarians are in the best interests of the animals and the successful completion of the experiments. In responding to the range and diversity of regulations related to animal research, IACUC at different institutions have varied considerably in their judgments of similar or even identical experimental designs. However, the *Guide* specifically notes (p 26) that input from outside experts may be advisable or necessary for IACUC evaluation of protocols. The SRBR and SRS can provide assistance in this process (http://www.srbr.org/Pages/exec.aspx; http://www.sleepresearchsociety.org/). Although institutions that conduct research on vertebrate animals are understandably concerned with the adverse consequences of failing to meet regulatory guidelines, USDA inspectors and AAALAC site visitors have understood that deviations from standard housing conditions that have been approved as scientifically justified by the IACUC are permitted and do not constitute any violation of USDA regulations or the Animal Welfare Act. The SRBR and SRS conjointly provide the present document as a basis for accommodation consistent with the Animal Welfare Act's clear legislative intent and the Public Health Service policy on humane care and use of laboratory animals. This document is intended to provide institutions with recommendations to assure that animals used in sleep and biological rhythms research receive reasonable and appropriate care. Although these guidelines are often similar to those that prevail in other countries, investigators and regulatory bodies outside of the United States may require modification of these practices as appropriate for compliance in their particular jurisdiction.

Recommendations

Long-term maintenance in circadian and sleep experiments. Animals used in biological rhythm experiments must often be maintained under constant conditions without extraneous manipulations or treatments to characterize behavioral or physiological cycles. Statistical requirements for estimation of period and phase through use of power spectral, periodogram, or other analyses typically require recording a sufficient number of cycles, each of which may be 24 h or more in length. Furthermore, accurate assessment of the phase and period of a biological rhythm requires sampling of sufficient frequency to avoid aliasing.[5,32] Thus, the experimental design will dictate how frequently and over what interval samples must be collected or observations made.

Rigorous maintenance of constant conditions is essential to assessing the characteristics of a biological oscillator, including its period, phase, and amplitude. After an environmental

perturbation such as a light pulse or a shift of the phase of the light:dark cycle, animals may experience multiple transient cycles before stabilization occurs. This situation mimics jet lag and reflects the crucial coordinating role of central and peripheral oscillators in the control of multiple physiological functions.[1,21] Disruptions including cage changes or even replenishment of food and water can introduce artifacts that compromise the experiment (Figure 1). Therefore, animals should receive sufficient supplies of food and water to permit changes that are less frequent than those typically provided in the animal facility. Nevertheless, food, water, and animal health must be checked often enough to ensure wellbeing, and sanitization of water bottles and cages must be consistent with animal health.

The maintenance of animals under constant conditions, not only of light or darkness but also of temperature and humidity, is facilitated by use of safety alarms that can alert staff to a power failure, over- or underheating, and so forth. Such alarms should be connected to an emergency office so that any problems can elicit a quick response to protect the animals and ensure the continuity of the study. Some studies on seasonal rhythms—for example, those pertaining to torpor or hibernation—may call for deviation from the standard temperatures used in most experiments. These deviations are subject to approval of the IACUC, which should take into account the natural history and physiology of the species under study.

In some cases, food and water availability can have important entraining effects.[26] The scientific study of such entrainment may require restriction of food or water availability or modification of the composition of the diet. As for any experiments that involve withholding food or water, the IACUC should ascertain the necessity of restriction or deprivation before approving the protocol. The investigator should provide scientific justification to the IACUC that describes the potential confounding effects that require withholding food or water and should describe procedures that will protect wellbeing of the animals, such as monitoring of intake or body weight.[34] Exceptions to standards for USDA-regulated species are to be acknowledged in IACUC semiannual evaluations and USDA annual reports.

Sanitation and caging. As emphasized in the *Guide*, animal health clearly depends on adequate sanitation. Changes of bedding often occur at weekly (or even more frequent) intervals in many animal facilities, as recommended by the *Guide* (p 69). The *Guide* recognizes, however, that research objectives may require a decrease in the frequency of changes of cages or bedding (p 70). This modification is often called for in circadian studies, in which disruption of the animal's environment is an inevitable consequence of the cage change. Stimulation of locomotor activity can not only contaminate data but also shift the phase of a circadian rhythm. Indeed, merely providing hamsters with a novel running wheel is sufficient to alter the circadian phase, given that activity provides a feedback effect on the biological oscillator.[3,20] Fortunately, a sanitary environment is possible even with reduced frequency of cage changes through use of a highly absorbent bedding that is made of materials that control the buildup of ammonia and other waste products (for example, Bed-O'-Cobs, The Andersons, PharmaServ, Framingham, MA). The use of such bedding is preferable to maintaining animals in wire-bottomed cages, which are discouraged by the *Guide* and have adverse effects on animals. An important consideration, however, is the effect of corncob bedding to decrease slow-wave sleep, possibly because animals find it a less comfortable bedding.[22] As an alternative, smaller rodents such as mice can be maintained in larger cages typically used to house rats in order to reduce ammonia buildup and the need for cage changes.

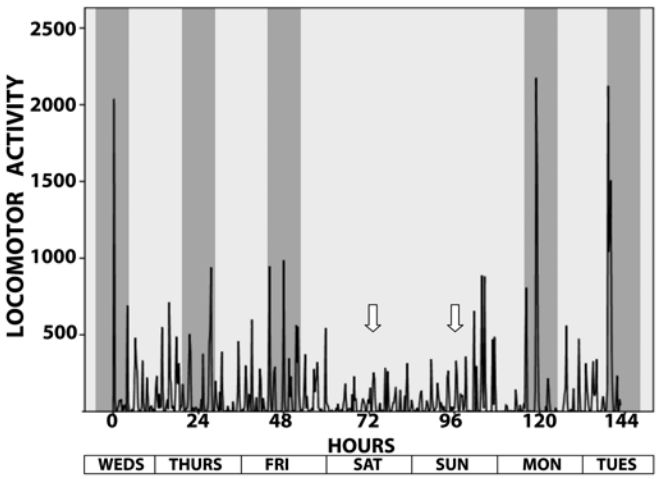

Figure 1. Representative home cage activity recording (infrared beam breaks) of a hamster housed in constant lighting conditions over a 6-d period. Dark gray bars indicate when animal care staff was present in the vivarium. Animal care staff entered the room and generated noises in vivarium such as cage washing, cage changes, banging of clean carts against walls in hallway and neighboring rooms, banging of doors, discussions, and so forth. These disturbances are most likely the source of the increased activity, which appears on the activity record as an artifact. On weekends, only the investigator and his staff entered the rooms quietly to check the animals (arrows). JC Walton and RJ Nelson, unpublished observations.

Another modification is to schedule cage and bedding changes at a time when animals are already active, to avoid provoking activity during the rest interval and thus introducing artifacts into the results. The optimal timing of cage changes can best be defined through consultation between the animal care staff and the investigator, who can determine when the animal is active by inspecting the activity record or other rhythms associated with activity and wakefulness. When a large number of animals is maintained in constant conditions for a particular experiment, the active periods of the experimental subjects may be distributed across the entire 24-h cycle, making it impossible for husbandry to occur at a single time of day without compromising an experiment. For these reasons, many IACUC have approved individual investigators for conducting cage changes and animal husbandry rather than relying on the institution's animal care staff. In such instances, the investigator may be expected to document husbandry activities to assure the IACUC that the standard of care is being maintained.

To isolate animals from unplanned perturbations that could disrupt circadian rhythm experiments, they often are housed inside larger cabinets that allow rigorous control of the photic environment. These enclosures must meet the requirements of cage space and air changes specified in the *Guide* and should be constructed of materials that are impervious to moisture, easily cleaned, and unlikely to harbor vermin. Maintenance of an environment suitable for such studies is facilitated by location of experimental rooms at some distance from sources of noise, such as elevators, cage-washing areas, and areas of heavy pedestrian traffic. Routine activities going on elsewhere in animal facilities can provide entraining cues to animals that are supposed to be in constant conditions. Some experiments may benefit from masking such extraneous noises by placing a white-noise generator in the animal room and setting its output at a level sufficient to prevent disturbance from events occurring in the animal facility. However, such devices typically are designed for the human range of hearing and may be ineffective if their output frequencies differ substantially from that of the

animal species under study. Therefore, locating experiments in areas apart from the central vivarium may be the most practical solution.

Illumination. The *Guide* recognizes the importance of illumination in the microenvironment (p 47); includes considerations of photoperiod, intensity, and spectral quality; and specifically mentions the importance of intrinsically photosensitive retinal ganglion cells which, along with rods and cones, mediate the entrainment of circadian rhythms in mammals. The spectral sensitivity of the retina of the species used in an experiment will determine the conditions under which they may be housed; many rodents are relatively insensitive to wavelengths above 600 nm, such that red light (provided that it is sufficiently dim) may be left on constantly in the animal room to facilitate observation by human caretakers.[14] Although visual observation is not necessarily easy at such wavelengths, the cone composition of the human retina may allow limited visibility through use of red light that the animals cannot see and which will not affect entrainment or free-running period. Caution must be exercised as brighter light, even if monochromatic and of long wavelengths, can have unintended effects on biological rhythms.[25,31,43] The color of translucent plastic rodent cages may affect light penetration and spectrum and thus influence biological rhythms of physiologic measures.[6]

The *Guide* also states (p 149) that attention should be given to aberrant visual cues, especially in circadian studies. This point deserves further discussion, in that even brief disruptions (such as a brief pulse of light) can shift or disrupt circadian rhythms and alter sleep patterns (Figure 2). As discussed earlier, the need of circadian studies to maintain animals in constant conditions for prolonged periods of time may be inconsistent with the requirement for daily visual checks that has been instituted in some animal facilities. In most (if not all) such studies, however, the activity of the animal is continually monitored—often by computerized recording of locomotion, temperature, or other relevant variables. Wiring should meet electrical codes and should be inaccessible to animals. Cables should be organized and bundled, and low-voltage devices should be used. Several systems are commercially available for such monitoring and are widely used by biological rhythm researchers. They afford the investigator and animal care staff the opportunity to confirm the health and wellbeing of animals continuously; entering the animal's enclosure is necessary only if remote measures indicate inactivity or show that physiologic variables are not in the expected range. Quite often, the apparent change in activity, body temperature, or other monitored variable is attributable not to a health problem but to a technical problem with the recording device, such as a broken wire, faulty switch, and so on.

When visual checks are necessary to establish the animal's health, they can often be accomplished without disrupting the experiment. When animals are in constant darkness, an infrared viewer (for example, Find-R-Scope, FJW Optical Systems, Palatine, IL) can be used to confirm animal welfare. A daily log of such checks is required to document the performance of required monitoring. Such arrangements have been followed successfully in many animal care facilities and are accepted as sufficient to comply with the *Guide* and the regulatory requirements.

As mentioned earlier, emotional states and mood are among the cognitive and physiologic parameters influenced by the circadian system.[19] In addition to their potential clinical importance, such findings may have implications for the welfare of animals in circadian experiments that require manipulation of photoperiod or lighting conditions. For instance, evidence suggests that rats kept in prolonged constant darkness experience increased apoptosis of monoaminergic neurons, reduced catecholaminergic input to the forebrain, and depression-like behavioral effects.[9,11] Maintenance of mice in constant darkness for 4 wk reduces neurogenesis in the hippocampus and activates cytokine (particularly IL6) expression.[27] However, unlike the controls that experience light and darkness, the animals that were exposed to constant darkness in these experiments were not tested at any consistent circadian phase, so there is concern about confounds in these attempts to establish effects of the light:dark environment on depression. Perhaps more important, it is not clear from these experiments whether darkness itself, as compared with lack of circadian entrainment, is responsible for neurological or behavioral effects. In Siberian hamsters, a seasonal species, naturally occurring fluctuations of daylength alter hippocampal cell complexity and modulate behavioral responses that have been associated with depression-like states.[47] Such changes may reflect a normal physiological response to prolonged darkness. However, evidence linking prolonged exposure to constant darkness with neurological or behavioral effects is not definitive in its current state; the available information remains subject to interpretation and additional investigation is needed. However, maintaining nocturnal rodents in constant darkness for many weeks does not lead to physical deterioration (as evidenced by body weight or condition) or increase stress (as indicated by adrenal activation).

Environmental enrichment. The recent revision of the *Guide* emphasizes the benefits of environmental enrichment to provide sensory and motor stimulation (p 52). Studies on circadian rhythms often include measurement of locomotor activity, which provides the opportunity to assess exercise. In fact, many such studies on biological rhythms offer animal subjects either ad libitum or temporally restricted access to a running wheel. Rodents of many species voluntarily make extensive use of the opportunity to run, and this option may mitigate concerns about the effects of a nonenriched microenvironment. Wheel running may have a stereotypic appearance but is dissimilar from the adverse stereotypies shown by caged animals in unenriched environments. Although running wheels typically are provided in cages whose area meets the space recommendations in the *Guide*, providing animals with the opportunity to run in a wheel allows extensive exercise that otherwise could only be gained by offering them a much larger cage. Equally important, mice provided with a running wheel for exercise show lower corticosterone production and fewer physiologic measures of stress and immune response, particularly when provided with a calm environment.[13] Thus, the exercise provided by running-wheel access in circadian rhythm studies may mitigate concerns raised about applicability of results garnered from use of sedentary laboratory rodents.[24]

Basic environmental enrichment for rodents (nesting material, toys, and so forth) can often be easily incorporated in studies involving long-term sleep recording. The use of a recording cable can sometimes limit or preclude the type of items that can be placed in the recording cage for enrichment (for example, small enclosures). Similarly, in some studies, cannulae or dialysis probes must be affixed to the animal for sample collection or recording purposes and generally preclude the presence of a running wheel. Sometimes other exercise devices can be offered, including running discs, but these are not always consistent with the purposes of the study. Experiments in which metabolic rhythms are studied often cannot include running wheels or other exercise devices. In such cases, the investigator should

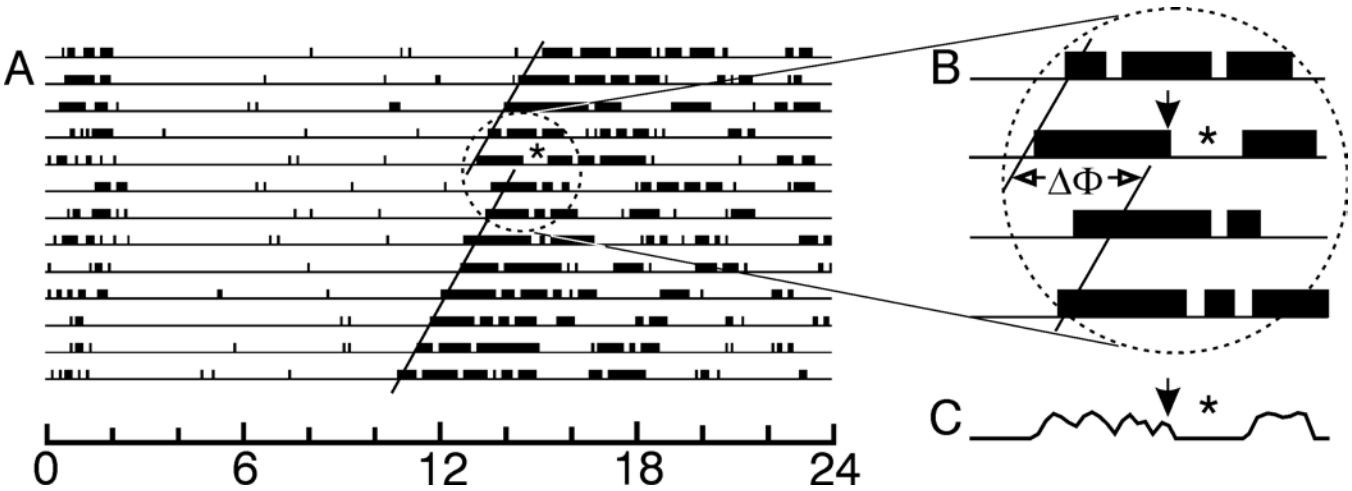

Figure 2. Light flashes of millisecond duration alter locomotor activity and circadian phase in mice. (A) Running record of a mouse shown in standard actogram format. Each line of the record represents 24 h; successive days are represented below one another. Mice had ad libitum access to a running wheel; revolutions produce black bars on the record. The mouse was maintained in constant darkness, except for brief interruptions as indicated by asterisks. Note that activity onsets occurred slightly earlier on successive days, indicating that the period of the endogenous rhythm was slightly shorter than 24 h. On day 5 of the experiment, the mouse was exposed to 10 flashes of 2 ms each, equally spaced across a 5-min interval during the early subjective night. The light stimulus produced a significant delay in the phase of the locomotor rhythms, as indicated by the rightward displacement of the line fitted to the activity onsets. (B) The response of the mouse is enlarged; the shift of circadian phase ($\Delta\phi$) indicated by open horizontal arrows reflects the delay in the timing of arousal induced by the light flashes, as measured by the linear regression fit to activity onsets. (C) Wheel running over the 5-min bin shown in panel B is plotted. Reprinted with permission from reference 29.

explain the scientific justification for the housing conditions and the relatively sedentary nature of the animal's habit.

Although a diverse, varied, and nonstandardized environment may have benefits for animal subjects, it can increase variability of results and lead to discrepancies between findings in different laboratories. This inconsistency can lead to an increase in the number of animals used, both to overcome the higher variability in results within an experiment and to resolve discrepancies between laboratories that would not otherwise occur. This situation runs counter to the guiding principle of reducing (whenever possible) the number of animals used in an experiment. The study cited in the *Guide* as contradicting this concern is a cursory comment[46] that pertains to particular behavioral measures in specific mouse strains within an individual laboratory. Thus a detailed evaluation of the benefits and costs of enrichment is important. Scientific concerns about adverse consequences of nonstandardized attempts at environmental enrichment deserve serious consideration, not just in studies of biological rhythms, but in other areas as well.

The *Guide* encourages caging of animals in social groups where appropriate. This practice is often incompatible with experiments on circadian rhythms and sleep because the behavior of individual subjects must be monitored. If activity is measured, it may be difficult (if not impossible) to determine which group-housed animal is making use of the opportunity to run in a wheel. Even when physiologic variables such as body temperature are measured by telemetry, most devices fail to discriminate between the signals sent by transmitters implanted from different subjects within the same cage. Furthermore, whatever their beneficial effects on health, social interactions constitute a relevant stimulus that can affect the phase and period of biological oscillators.[4] As a result, social housing is not compatible with experimental design and interpretation in many studies of biological rhythms.

Conclusions

Experiments on biological rhythms and sleep may call for conditions of animal maintenance, observation, sanitation, and environmental conditions that differ from those that are routine in animal facilities and the standard practices in the *Guide* or the Animal Welfare Act. Such nonstandard housing and care is compliant with regulations, provided that it is explained and scientifically justified by the investigator and thus approved by the IACUC. The investigator should consult with the attending veterinarian and the IACUC regarding animal welfare in such experiments and implement procedures that permit adequate assessment of animal health and welfare. Investigators may train members of the animal care staff or accompany them in such monitoring to ensure that animal welfare is verified and recorded during the course of experiments on biological rhythms.

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