INSTRUMENTATION & TECHNIQUES

Sleep, activity, temperature, and performance: Techniques for long-term monitoring in the mouse

PIERRE M. HAHN, TOM SCHMIDT, AJAY AGARWAL,
RICHARD FRIEDMAN, and MERRILL M. MITLER

State University of New York at Stony Brook, Long Island, New York 11794

This paper describes a test chamber, a food acquisition task, and a training procedure for the long-term testing of an implanted mouse in a circadian rhythm experiment. For the training phase, a special-purpose controller is provided to a single cage. For the experimental phase, behavioral, electrophysiological, and environmental measures are collected by means of a mini-computer.

There is a growing body of clinical data indicating that disruptions in circadian rhythms cause many types of discomfort and malaise (Rockwell, Winget, Rosenblatt, Higgins, & Hetherington, 1978). One of the most common problems is sleep interrupted with many wake periods.

In a study of the relationship between activity rhythms and sleep rhythms, Mitler, Lund, Sokolove, Pittendrigh, and Dement (1977) described a disorganization in the sleep-wake rhythm of mice that were isolated from cues to terrestrial time. This disorganization was characterized by a large reduction in the amplitude of the sleep-wake rhythm (measured in minutes of sleep per .5 h) that was not associated with any abnormality in the activity rhythm (measured as presence or absence of wheel running for each .5 h). These disorganizations emerged under constant darkness, constant light, and under 12-h-light/12-h-dark conditions. The cause of these sleep-wake abnormalities may have been an inability of the sleep-wake rhythm to remain in the same temporal relationship with other rhythms, such as activity (Czeisler, Richardson, Coleman, Zimmerman, Moore-Ede, Dement, & Weitzman, 1981).

To more completely analyze these disorganizations in the sleep-wake rhythm, techniques for the continuous electrophysiological monitoring of mice in isolation have been combined with techniques for assessing

behavior. Specifically, the development of a technique for monitoring instrumental activity associated with food acquisition as well as locomotor activity allows for the study of the interrelationships of multiple circadian rhythms and the assessment of consequences of disruption in circadian rhythm on the biologically relevant task of food acquisition.

The development of an isolated monitoring environment suitable for long-term circadian studies in rodents involves a number of methodological problems. In this report, we describe techniques for the training and continuous monitoring of an implanted animal for sleep and wakefulness, brain temperature, wheel running activity, performance on a discrimination task that provides the animal's daily food, and environmental measures.

THE ENVIRONMENT

The mouse's housing is designed to permit long-term testing without disturbing the subject. The test chamber (Life Sciences Associates, Bayport, New York) contains upper and lower compartments (see Figure 1) that act as noise dampers and are light tight. The upper chamber (42 cm wide, 30 cm deep, and 32 cm high) encloses the 6-mm-thick Plexiglas cage, which is 21 cm wide, 24 cm deep, and 11 cm high. The cage contains the behavioral panel (right-hand wall), a running wheel (left-hand wall), an eight-wire mercury commutator (in the ceiling), an opening door (front), and a floor, which is formed by the upper portion of the housekeeping drum.

The behavioral panel contains three 16-mm-diam holes, approximately 3 cm from the floor, spaced 4 cm

This research was supported in part by NINCDS Grant NS 15787. P. M. Hahn, R. Friedman, and M. M. Mitler are with the Department of Psychiatry and Behavioral Science and the Long Island Research Institute; T. Schmidt is with the Department of Psychology; A. Agarwal is with the Department of Electrical Engineering.

Figure 1. Line drawing of the "mouse housing" showing schematically (A) the commutator, (B) the behavior panel, (C) the running wheel, (D) the cleaning drum, and (E) the pan for the cleaning solution.

apart, which are centered on the wall. Each hole is a nose switch with an infrared light-emitting diode (LED) and sensor that logs a response when the nose protrudes at least 10 mm into the hole. Each switch has a yellow-colored LED that can be used as a discrimination stimulus during training.

The running wheel (13 cm on the outer edge and 8 cm in the back) is slanted so that the animal can use it without the wheel's damaging the wire bundle connected between the implant and the commutator.

The ceiling holds an eight-wire commutator, two small incandescent lights, a speaker, and a microphone. The mercury-filled commutator has spherical ball bearings to support the connector. The static force necessary to start turning the commutator is less than .1 g-cm. The incandescent lights control day/night lighting, the speaker is for tone presentation, and the microphone records ambient noise.

Inside the lower chamber (60 cm wide, 40 cm deep, and 60 cm high) is the cleaning drum (a cylinder 50 cm in diameter and 25 cm wide). The uppermost portion of the drum serves as the floor of the animal's cage. The

lower portion dips into a cleaning solution, which is changed continuously (not shown in Figure 1). Rotation of the cleaning drum is controlled by the actions of the animal: The drum rotates (10 deg/min) only when the mouse is turning the running wheel.

Two feeders, for redundant operation, are mounted on top of the enclosure with a common dispensing tube ending about 4 cm above the center hole. Each feeder has a sensor that monitors when a pellet has been dispensed. Water is available ad lib from a lick dispenser on the back wall.

An exhaust fan pulls air out of the enclosure through a light trap at the bottom of the lower chamber. Air enters at the top through the feeding tube dispenser hole.

The electronic circuitry necessary to energize and sense is mounted on the outside of the chambers for accessibility.

THE FOOD ACQUISITION TASK

Mice (C57 B1/10J) are trained to perform a temporal duration discrimination task to obtain food. Temporal duration discrimination tasks measure the ability of a subject to use stimulus duration (usually a tone) as a cue signaling which of two responses is correct in a simple choice task. We have modified a procedure employed by Roberts and Church (1978), who used the technique to demonstrate the properties of a hypothetical biological clock that permitted rats to make discriminations of short intervals. We are taking advantage of the ability of the mouse to make these same discriminations in order to examine the properties of the biological clock (long term, circadian) when perturbed by constant conditions.

Subjects are presented with the array of three response holes arranged side by side along one wall of the test chamber. In order to obtain a food pellet, the mouse must carry out two responses in the appropriate sequence, both spatially and temporally.

A typical trial consists of the following sequence of events: The mouse pokes his snout into the center hole. This response initiates the trial, causing the production of a tone of predetermined length. Following the offset of the tone, the animal is required to respond to either the left or right hole, with the correct response determined by the duration of the tone. For example, tones with a duration below a particular value signal a reinforcement following a left-side response; tones above that value signal a reinforcement following a right-side response. Typically, only four tones are employed; two have durations above criterion and two have durations below.

DATA COMMUNICATION

During the training phase, to partially automate the training, the cage is connected to a trainer system.

Subsequently, when the animal is implanted and is trained adequately to obtain all daily food, the cage is connected to the communication interface with the on-line computer for data gathering.

THE TRAINER

The trainer has five modes of operation, each of which can be modified by means of specific switches. The modes are the following: Manual—This mode may be operated from both a pendant control and/or the system. Pushbuttons allow presentation of tone and reinforcement at the experimenter's discretion, independent of the subject's behavior. Mode 0—A response to any of the three holes results in a food pellet presentation. Mode 1—Only center-hole responses result in a food pellet presentation. Mode 2—One tone length is introduced. A trial is initiated with a center-hole response, which starts the tone, and either a left- or a right-hole response results in a food pellet presentation. Mode 3—More than one tone length is introduced. This mode is similar to Mode 2, except that the mouse must discriminate tone lengths and decide on the correct hole response to obtain a food pellet presentation.

For each mode, there are several selectable options. Eight tones, two intertrial intervals (ITIs), correct trial ITI and incorrect trial ITI, and one default time are presettable using thumbwheel binary-coded decimal (BCD) switches in the range of .1 sec to 199 sec. The sequencing of the 8 tones is programmable and recycles after 64 tones. Four tone lengths are associated with a right-hole response being correct and the other four with the left-hole response being correct. To allow maximum flexibility during training, the experimenter also has the following added capabilities with two-position switches: (1) Response during tone (incorrect/allowed) is selectable in Modes 2 and 3. (2) Default time (active/inactive) is selectable in Modes 2 and 3. (3) Termination of the trial by an incorrect response (active/inactive) is selectable in Modes 2 and 3. (4) Cue lights (active/inactive) are selectable in any of the modes for any of the response holes. In addition, selection of only the correct response to be cued (right or left hole) or both holes to be cued is available. The brightness of the cue lights is adjustable.

ON-LINE COMMUNICATION INTERFACE

A communication interface allows up to six cages to be connected via an asynchronous ASCII line to the computer, but at this time the speed of the minicomputer allows us to use no more than four cages simultaneously. Each cage has a small processor that controls the behavioral task and the day/night cycle and formats the data in 30-sec epochs that are available to the computer at any time. The common interface controls the "mouse" time of day and has 16K bytes of local memory. All hardware is battery powered. An ac power failure lasting longer than 40 min would overflow the 16K-

byte memory and some data would be lost. As a backup, a 14-channel FM tape recorder is used for storage of the behavioral, environmental, and electrophysiological data.

PROCEDURE

Several mice have been trained to reliably discriminate between two sets of tones: a pair of shorter tones, 2 and 4 sec in length, and a pair of longer tones with durations of 6 and 12 sec. While the exact procedures used to train each animal have varied slightly, the experience gained has resulted in the formulation of a standardized protocol. Certain aspects of the experimental situation remain constant throughout.

(1) Response—The mouse must insert its snout (or some other body part) a distance of at least 10 mm into one of the holes. The snout interrupts an infrared light beam and, after a time delay of 1/8 sec to account for "switch bounce," a response is logged. Offset of the response has a similar time delay.

(2) Correct Response—Temporally, the correct response can occur only after tone offset and before the end of the default time. Spatially, the correct response is to the peripheral hole (right or left) associated with the tone length.

(3) Scoring of Errors—Incorrect responses are scored when (a) the mouse responds to the correct hole during a tone, (b) the mouse responds at the wrong hole following tone offset, (c) the mouse responds at the wrong hole during a tone, and (d) the mouse fails to respond during the 10 sec default period following tone offset.

(4) Temporal Distribution of Trials—The ITI is maintained at 30 sec. Timing of the ITI is initiated at the end of the tone, following any response after the tone or at the end of the 10-sec default period following tone offset if there were no responses.

(5) Cue Lights—Small lights are located inside each of the three holes. Cue light "on" at the center hole indicates that a response will initiate a tone. The cue lights in the side holes come on following tone offset. They may come on separately or in tandem, depending on the training condition in effect. Use of the cue lights acts to reduce the occurrence of responses during ITI. The intensity of the cue lights can be varied, and the cue lights can be turned off for the final phase of the study.

Pretraining for the tone duration discrimination task takes the following form.

(1) After reduction to 80% of its free-feeding weight, the mouse is introduced into the test cage. In order to obtain a food reinforcement (45-mg P. J. Noyes precision food pellet), the mouse must first initiate a .5-sec tone by responding at the center hole. Following tone offset, a response at either side hole is rewarded. Response during the tone results in the termination of the trial and the initiation of the 30-sec ITI. Mice rapidly master this initial task.

Over the next several days, tone length is incremented

to 1.0, 2.0, 3.0, 4.0, and 5.0 sec. The rate at which tone length is incremented is dependent on the behavior of the mouse. A stable correct performance level of at least 65% over two 24-h sessions is required before advancement to the next tone. Once stable performance has been established at the longest tone duration, the next stage of training is begun.

(2) At this stage, the discrimination task is introduced. The mouse must respond at the appropriate side hole following tone offset. The appropriate response is indicated by the cue light inside the hole. Again, advancement to the next stage of training is determined by the mouse's performance, with a criterion of 65% or better correct responses necessary.

The actual sequence of tone lengths presented during this stage varies. Mice have a tendency to develop position preferences during the first stage of training, so biasing to the opposite side may be necessary initially. However, once initial position preferences are overcome, a randomized sequence of tones is presented.

(3) In the next phase, both side cue lights come on following tone offset (eliminating the discriminatory value of the visual cues). Tones are presented in the sequence 20 long, 20 short, 20 long, 2 short, 2 long. Again, advancement to the next stage is dependent on performance at the criterion of 65% correct.

(4) This is the final stage and is the actual tone duration discrimination task. The procedure is identical to Stage 3, except that the tones are presented in a randomized sequence.

Once a mouse learns to feed itself on at least a two-tone task, it is implanted for electrocorticogram (ECOG) and electromyogram (EMG) recording using the surgical method of Mitler and Levine (1970). In addition, a thermistor (Thermometrics, Edison, New Jersey, Type A02C9-BR14KA153N-C) is implanted in contact with the dura mater through a burr hole in the skull. The leads are formed into an eight-conductor shielded cable that is connected to the eight-ring mercury commutator in the cage roof.

ECOG and EMG signals are acquired and analyzed on-line by a small computer (DEC PDP-11/03). A computer program labeled SQUEEK (Vincent, 1976;

Vincent, Dement, & Mitler, 1977) is taught to discriminate wakefulness from rapid eye movement (REM) and non-REM (NREM) sleep. SQUEEK uses a self-modifying template-matching algorithm to discriminate the various states and can operate continuously (in real-time) with little or no retraining for months at a time. SQUEEK has been modified to allow the behavioral data to be used in the decision making paradigm, thereby forming an array of 30 sec by 30 sec status on the animal.

It is usually necessary following surgery to provide additional behavioral training sessions while the animal is recovering.

Once the animal is fully recovered from surgery and exhibits stable feeding on a two-tone task, it enters the experimental protocol.

REFERENCES

- CZEISLER, C. A., RICHARDSON, G. S., COLEMAN, R. M., ZIMMERMAN, J. C., MOORE-EDE, M., DEMENT, W. C., & WEITZMAN, E. D. Chronotherapy: Resetting the circadian clocks of patients with delayed sleep phase insomnia. *Sleep*, 1981, 4, 1-21.
- MITLER, M., & LEVINE, R. Sleep analysis and a simple technique for deprivation of low-voltage, fast-wave sleep in a species of deer mouse, *P. m. bairdi*. *Psychophysiology*, 1970, 7, 112-120.
- MITLER, M., LUND, R., SOKOLOVE, P., PITTENDRIGH, C., & DEMENT, W. Sleep and activity rhythms in mice; descriptions of circadian patterns and unexpected disruptions in sleep. *Brain Research*, 1977, 131, 129-145.
- ROBERTS, S., & CHURCH, R. M. Control of an internal clock. *Journal of Experimental Psychology: Animal Behavior Processes*, 1978, 4, 318-337.
- ROCKWELL, D. A., WINGET, C. M., ROSENBLATT, L. S., HIGGINS, E. A., & HETHERINGTON, N. W. Biological aspects of suicide: Circadian disorganization. *Journal of Nervous and Mental Disease*, 1978, 166, 851-858.
- VINCENT, F. A. SQUEEK—A computer algorithm for the real time automatic sleep state scoring of mouse electroencephalogram. Unpublished master's thesis, Stanford University, 1976.
- VINCENT, F. A., DEMENT, W. C., & MITLER, M. M. A computer algorithm for the real time automatic sleep state scoring of mouse electroencephalogram. *Sleep Research*, 1977, 6, 216.

(Received for publication June 17, 1981;
revision accepted September 17, 1981.)