Proc. Natl. Acad. Sci. USA
Vol. 75, No. 12, pp. 6276–6280, December 1978
Neurobiology

"Dark-active" rat transformed into "light-active" rat by destruction of 24-hr clock: Function of 24-hr clock and synchronizers

(circadian clocks/biological clocks/suprachiasmatic nuclei/nocturnal and diurnal patterns/brain lesions)

CURT P. RICHTER

Department of Psychiatry, Psychobiological Laboratory, Johns Hopkins Medical School, Baltimore, Maryland 21205

Contributed by Curt P. Richter, August 24, 1978

ABSTRACT **In alternating 12-hr periods of light and dark the rat is active mainly in the dark. Its activity in the dark (beginning at 1800) depends exclusively on release of activity by the 24-hr clock. In the light (beginning at 0600) the 24-hr clock inhibits activity; the normal rat becomes totally inactive in the light except for activity resulting from external stimulation. After section of the connections between the optic chiasma and the hypothalamus, some rats become totally and permanently inactive in the dark. This sectioning destroys the 24-hr clock. After destruction of the clock removes inhibition of activity in the light period, the rat becomes active promptly at start of the light period—i.e., becomes a "light-active" animal. In the normal rat, activity becomes synchronized to start of the dark (by the electric clock at 1800), regardless of the amounts of activity. Destruction of the 24-hr clock eliminates the synchronizer at 1800. However, almost at once, activity, eating, and drinking are kept together by a second synchronizer, start of the light (by the electric clock at 0600). This may explain the ability of the rat to survive after destruction of the 24-hr clock.**

The wild Norway rat spends most of the day sleeping or hiding in deep burrows or other hideouts; it spends most of the night on the outside searching for food, water, and mates. It has several anatomical characteristics of "dark-active" animals: small eyes, a high proportion of retinal rods, and very small optic nerves.

Results of the following experiments showed that sectioning of the connections between the optic chiasma and the hypothalamus in certain cases changed the dark-active rat into a "light-active" animal. The lesioned rat spent most of the day in activity and all of the night in sleeping or total inactivity. These results made it possible: (*i*) to determine in more detail the function of the 24-hr clock during the dark and light; (*ii*) to outline the role played by a synchronizer of functions in the normal animal; and (*iii*) to describe a replacement synchronizer in animals deprived of their 24-hr clocks.

METHODS

Cages and Recording Devices. Spontaneous running activity in revolving drums (1, 2) was the main measurement for these experiments. Each cage contained a revolving drum on one side of a central partition and a living compartment (with food cup and graduated 100-ml water bottle) on the other. A cyclometer recorded revolutions of the drum, and an eccentric cam and microswitch registered single revolutions on an operation recorder. Five stands of 16 cages each were used for 80 animals. The 16 cages on one stand also recorded drinking times (3). The rats used in these experiments were randomly distributed on these five stands.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Conditions. The laboratory remained totally dark for 12 hr (1800–0600) and well illuminated with Sylvania F40D fluorescent bulbs for 12 hr (0600–1800). Room temperature remained fairly constant (23–24°C) throughout the year. The laboratory remained quiet during the 12-hr dark period except for occasional animal squeaks or noises made by turning of activity drums, etc. A short burst of noises—sneezes and rustling—occurred when the lights came on at 0600. On weekdays, laboratory personnel appeared at 0800 and withdrew at 1500; on weekends the times were 0700 and 1100. During the day, distant noises came from the hospital corridors and courtyard. Daily routine included collecting records of running activity and food and water intakes and making vaginal smears. Readings of body weight were made weekly.

All conditions remained constant over the 12-yr experimental period (1963–1975). Laboratory personnel remained the same.

All of the domesticated rats used in this study came from my colony (started 55 yr ago). A total of 264 rats were used for the operations and 40 unoperated rats were used for other parts of the study.

Instruments. In addition to the fine ophthalmological scalpel, wire (very thin) loops (2–3 mm) and bayonets were used to make the lesions. The loop stem was soldered to a rod that fitted into the chuck of a dental drill for spinning either by hand or motor. The bayonet [modified from one used by Halász (4)] consisted of a knife, a stem enclosed in a tube, and a handle for rotating the knife; a clamp secured the tube to a stereotatic instrument. Bayonets had razor-sharp edges from tip to stem.

DeGroot coordinates (5) located some of the lesions; my own coordinates (base of skull in a horizontal plane) located the others. The text will supply other information about the operations. Moore's recent review (6) gives a good account of the development of knowledge about this part of the brain, particularly about the suprachiasmatic nuclei.

Autopsies. At autopsy, endocrine glands and main organs were weighed and fixed in formalin. The ventral surface of the freshly removed brain was inspected for a darkened area near the optic chiasma and then photographed. In some instances the brain was removed and fixed in formalin; in others it was removed, frozen, and cut in 25-μm sections (7) for immediate microscopic study for location of the lesions.

Records. During the entire length of these experiments (in many instances a year or more for each rat), three types of records were kept for each rat. One plotted daily readings of running activity and food and water intakes and weekly records of body weight, along with operative notes, special observations, and autopsy findings; the second consisted of photographic prints of daily 24-hr records of distribution of activity; and the third contained photographs of brain sections.

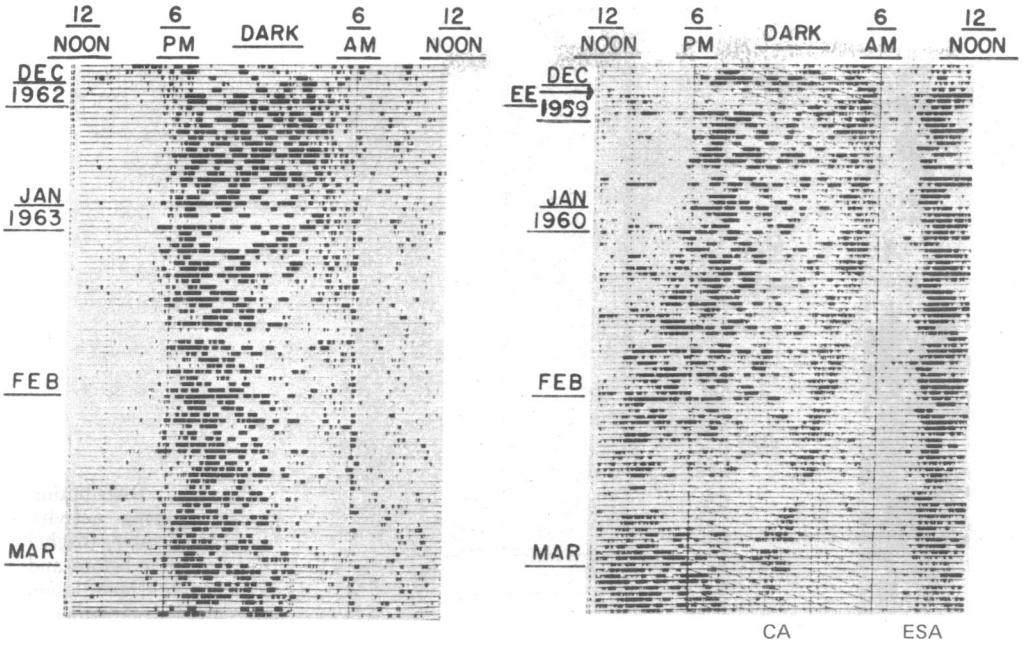

FIG. 1. Activity-distribution records, shown as noon-to-noon with succesive days appearing one below another. (*Left*) Normal rat, showing restriction of activity almost exclusively to 12-hr dark period. (*Right*) Before and after blinding (EE), showing freeing of clock activity (CA) in the dark by blinding and absence of effect on external-stimulation activity (ESA) in the light produced by presence of laboratory personnel.

RESULTS

Control Observation. Fig. 1 *left* shows the activity-distribution record of a normal rat kept in alternating 12-hr periods of light and darkness. This rat became active at 1800 (start of the dark period) and remained active throughout much of the dark period. In the absence of any external stimulation, it remained inactive in the light. In presence of external stimulation (noises and other disturbances), the rat may become very active (particularly if it is a very sensitive animal such as a wild Norway); Fig. 1 *right* shows the activity-distribution record of a normal rat before and after blinding. Before blinding, this rat showed the normal amount of activity limited to the dark but after blinding, onset of the daily active period occurred earlier each day with great regularity. This illustrates freeing of the clock by blinding and that all clock activity starts in the dark period. Of further interest here is that blinding had no effect on the considerable amount of activity in the light that resulted from external stimulation (appearance of laboratory personnel). Thus, for the normal rat, dark activity records clock activity.

Effect of Horizontal Lesion on Activity. *Scalpel lesion.* These experiments were started in 1963 with a search for a connection between the optic chiasma and the hypothalamus and in an effort to determine what behavioral manifestation might be produced by sectioning of such a connection. I first severed all possible connections by a horizontal cut between the chiasma and the hypothalamus with a very fine opthalmological scalpel through an exposure made by removal of the anterior third of the right hemisphere. Seventeen rats had this lesion; five survived and thrived (Table 1). In three rats, such lesions produced a remarkable and unexpected result—the dark-active rat at once became light-active, permanently remaining inactive in the dark and very active in the light. Fig. 2 *left* shows the activity-distribution record of one of these rats. On the day after the operation, the rat became almost totally inactive in the 12-hr dark period. After a few days, onsets of activity became entrained to 0600 when the lights came on. After that the rat remained almost totally inactive in the dark period and became very active beginning promptly at 0600, the start of the light period. It became inactive each day near 1500, at the time of departure of laboratory personnel, indicating that its activity had resulted in large part from external stimulation. Compar-

Table 1. Effects of experimental lesions

Method	Rats	Effect: Inverted	Doubtful	None
Knife	17	3	2	12
Loop	82	34	18	30
Bayonet (180°)	110	13	18	79
Bayonet (360°)	20	5	6	9
Electrolytic	35	8	5	22
Total	264	63	49	152

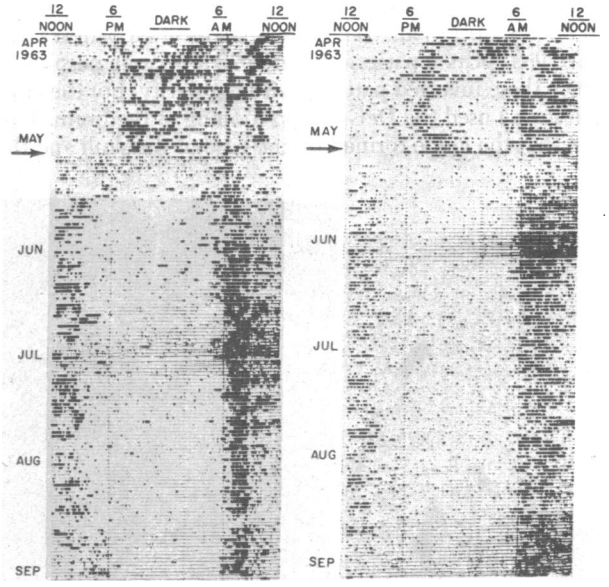

FIG. 2. Activity-distribution records of two rats before and after hypothalamic lesion (arrow shows time of operation). (*Left*) Horizontal cut between chiasma and hypothalamus with fine opthalmological scalpel. (*Right*) Lesion made by rotating 2-mm loop just above chiasma. These operations shifted activity from 12-hr dark period to 12-hr light period.

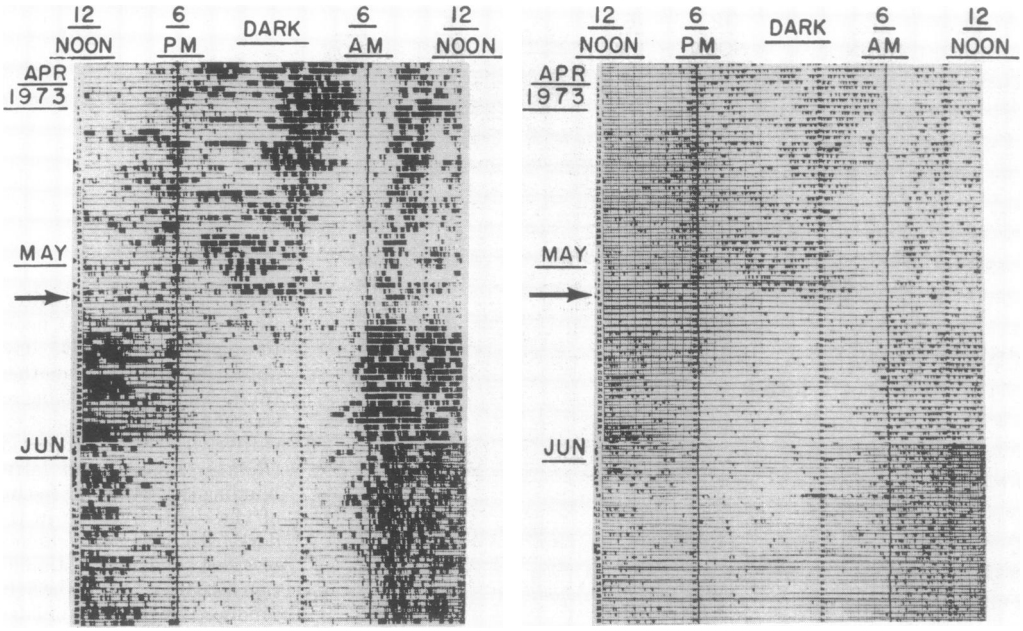

FIG. 3. Distribution charts showing activity (*Left*) and drinking (*Right*) times of a rat before and after a hypothalamic lesion (loop method, at arrow).

ison with records of normally light-active squirrel monkeys (8) showed close agreement—that is, the dark-active rat had become light active. Three of the five rats had definite inverted patterns—that is, they became active in the light and inactive in the dark (Table 1).

Loop lesion. In a further series of experiments, lesions were made with a loop introduced into the brain through the longitudinal sinus down to the optic chiasma at the base of the skull and spun several times by hand or motor. Some of the first lesioned animals showed records that closely resembled those seen after the scalpel operation.

Fig. 2 *right* shows the record of the first rat with a lesion made with a 2-mm loop spun through several full revolutions. This record closely resembled that of the rat with the scalpel lesion (Fig. 2 *right*), particularly in cessation of activity at time of departure of the laboratory personnel at 1500.

Of 82 loop operations, 34 gave definite inverted patterns and 18 gave less definite patterns; after 1972 it became possible to produce these inverted patterns with regularity. For the later loop lesions I used the DeGroot positioning of the brain. This meant that the angle formed by the base of the skull and the horizontal gave the loop a better approach to the suprachiasmatic nuclei on the optic chiasma.

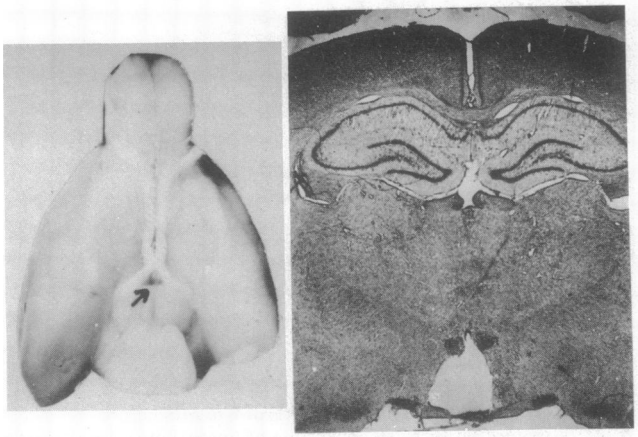

FIG. 4. (*Left*) Ventral surface of rat brain (rat from Fig. 2 *right*), showing darkened area just posterior to optic chiasma (arrow). (×3.) (*Right*) Coronal section at level of optic chiasma, showing absence of suprachiasmatic nuclei. (×3.)

Bayonet lesion (180° rotation). For this operation (partial island), I inserted the bayonet through the longitudinal sinus down to base of the skull, withdrew it 1 mm or less or not at all, rotated it 90° to the left and to the right several times.

Lesions made with a great variety of bayonets produced a few of the best inverted records. The rats became active promptly at 0600 and scarcely ever entered the activity drum in the dark over many months, and so they were practically totally inactive in the dark. Of 110 rats with bayonet (180°) lesions, only 13 showed inverted patterns.

Bayonet lesion (360° rotation). For this experiment (island), the bayonet was inserted to the base of the skull, withdrawn 1 mm or less or not at all, and rotated through 360°. The results gave a high percentage of pathological changes and few inverted records. Of 20 operations, only 5 produced inverted patterns of the type shown in Fig. 2. The rats became active promptly at 0600 and inactive near 1500.

Electrolytic needle. For these experiments I used insulated needles of various gauges bared at the tip for 1 mm. Current strength ranged from 2 to 3 mA and duration was 10–30 sec. I placed some of the lesions in the midline and some bilaterally by 1 or 2 mm. Some of the larger lesions gave good inverted records, whereas smaller lesions, both midline and bilateral, did not produce inverted patterns. Of 35 operations, 8 produced inverted patterns.

Activity, Drinking, and Eating Times. Results of my earlier studies (2, 9) had demonstrated a close correlation among activity drinking, and eating times—the three behavioral "hands" of the 24-hr clock. In the present study, drinking times recorded in 57 rats closely agreed with the distribution of activity in the lesioned rats. In rats with an inverted pattern, drinking times paralleled activity (that is, became predominant during the light period) (Fig. 3). It would seem likely, therefore, that feeding times also showed this same distribution.

Location of Lesions That Produced Inverted Pattern. With few exceptions, the lesions, however produced, that resulted in the shift from dark to light activity manifested themselves at autopsy on the ventral surface of the freshly removed brain by a darkened area just posterior to the optic chiasma (Fig. 4 *left*). Lesions in or near this area also showed up in sections

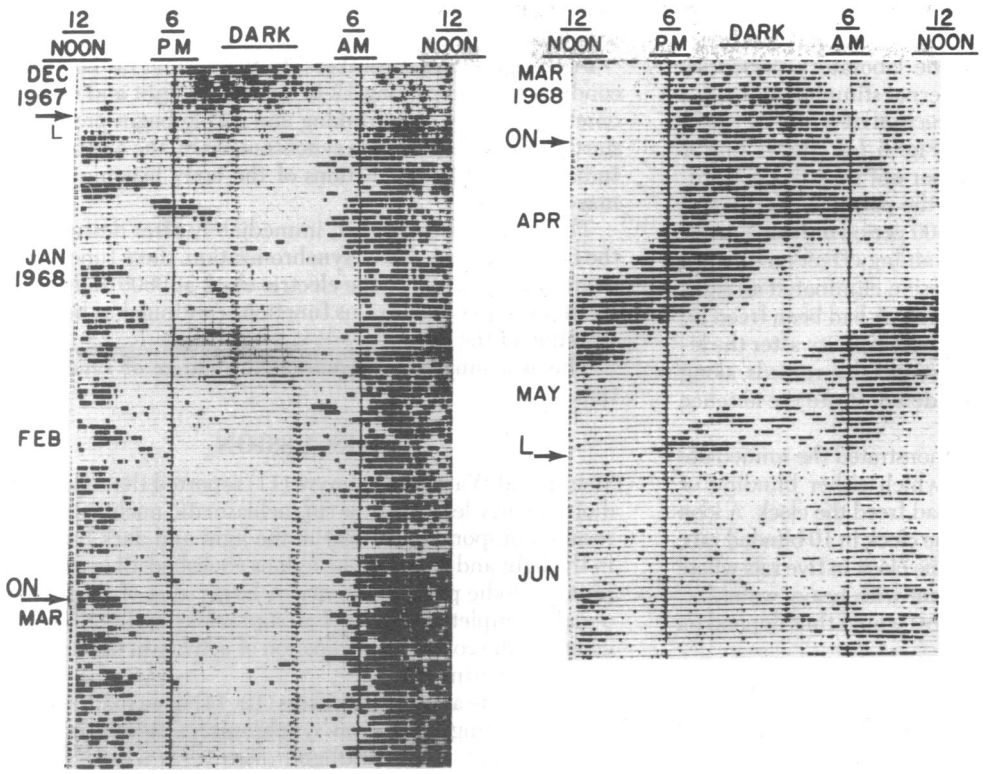

FIG. 5. (*Left*) Activity-distribution chart of rat before and after the hypothalamic lesion (L), showing the total absence of activity in the dark period and absence of all signs of presence of the 24-hr clock. (*Right*) Destruction, by lesion (L), of clock that had been freed by blinding (ON).

made from freshly frozen unstained brains or from injected, fixed, and stained brains.

A stained section (Fig. 4 *right*) from this brain, from one of the first rats with a loop lesion (see Fig. 2 *right*), shows destruction of the suprachiasmatic nuclei on the optic chiasma and of a large overlapping area. Most of the rats with inverted patterns showed similar lesions. None of the lesions, however, was limited strictly to the suprachiasmatic nuclei. Attempts to make smaller lesions by reducing size of the loops and bayonets failed to produce inverted patterns.

Thus, at present the findings indicate that appearance of inverted patterns depends on destruction of the suprachiasmatic nuclei and possibly also of some closely related structures. The retino-hypothalamic nerves may have been cut in some animals.

Inverted Pattern. Table 1 shows that 63 of 264 operations produced inverted patterns. Loop lesions produced them with the greatest consistency. These patterns have the following characteristics: (*i*) immediate and permanent total elimination of activity in the dark; (*ii*) immediate shift of all activity in the

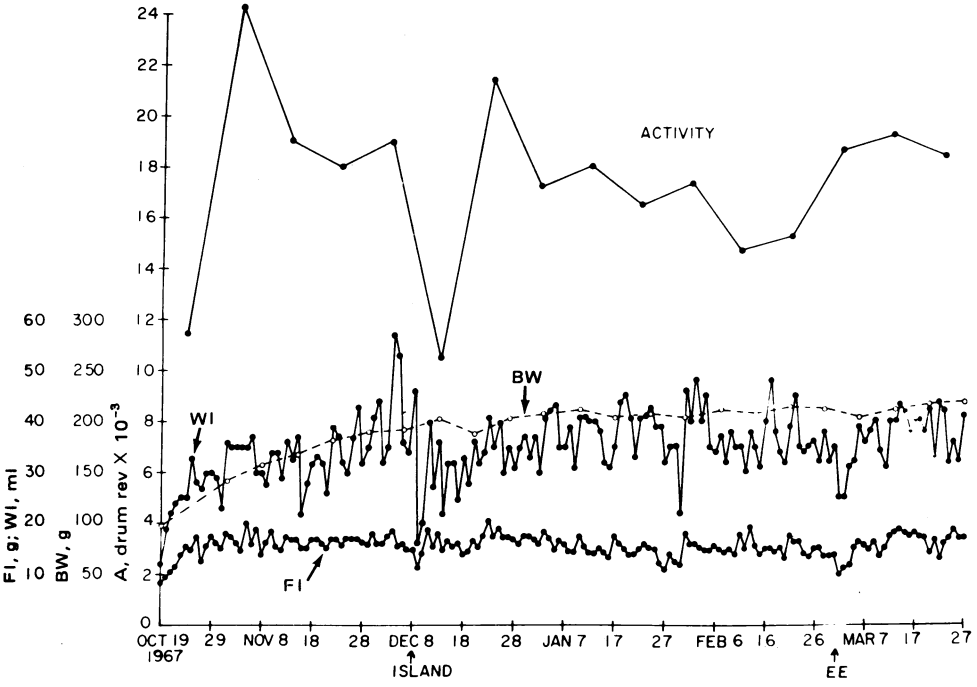

FIG. 6. Record of daily activity (A, shown as 10-day averages) and food (FI) and water (WI) intakes and weekly body weight (BW) of rat with inverted activity pattern after an island lesion (bayonet method).

dark (starting at 1800) to the light period; (*iii*) prompt start of active phases (at 0600) when the lights came on and continuation of activity until departure of the laboratory personnel (1500)—that is, after cessation of external stimulation.

Inverted patterns result from destruction of the 24-hr clock. The activity-distribution record in Fig. 5 *left* shows that a bayonet lesion (360°) produced a clear-cut inverted pattern. Blinding had no detectable effect on the patterns. The record also shows that activity in the 0600–1500 period did not depend on light (this period has a strong entraining effect on blinded rats). Fig. 5 *right* shows that a loop lesion eliminated all signs of presence of the clock in a rat whose clock had been freed by blinding. It also shows the entrainment of activity after the lesion to the 0600–1500 period. These remarkable records, along with all the other inverted patterns, demonstrate the absence of all clock activity.

Results of further experiments demonstrated the immediate destruction of the clock in rats in which either blinding or treatment with heavy water (^2H_2O) had freed the clock. A loop lesion totally eliminated all signs of the clock in 10 blinded rats. The lesion also eliminated freeing of the clock in five rats whose clock had been freed during treatment with heavy water.

The appearance of inverted patterns would thus depend on elimination of the 24-hr clock.

Rats with best inverted patterns showed least or no general effects of lesions. The activity-distribution records, showing presence or absence of inverted patterns, and the records of daily total running activity and food and water intakes and weekly readings of body weight made it possible to determine what effect the lesions had not only on activity-distribution but on the general metabolic condition of the animals. A survey of these two types of records showed that rats with good inverted patterns showed little or no general effects (Fig. 6). This record shows that the lesion that produced the great change in behavior (Fig. 5 *left*), the shift to light activity, had no detectable effect on any of the metabolic functions (total daily running activity, daily food and water intakes, and body weight). Fifteen animals had almost the same type of record. Clearly, the clock could not have had any effect on the precision of homeostatic functions of the animal (10).

CONCLUSIONS

On basis of results of the experimental studies, I have made the following conclusions regarding: (*i*) function of the 24-hr clock; (*ii*) how normal rats react after loss of their clocks; and (*iii*) function of synchronizers before and after destruction of the 24-hr clock.

Function of 24-Hr Clock: Dark Period. *In normal rat.* The clock releases activity in the dark.

After destruction of clock. The clock no longer releases activity in the dark; the rat becomes permanently and totally inactive in the dark.

Function of 24-hr Clock: Light Period. *In normal rat.* The clock inhibits release of activity in the light. The rat remains totally inactive except for activity resulting from external stimulation (chiefly from disturbances produced by laboratory personnel).

After destruction of clock. Freed from inhibition, animals now follow internal urge to compensate for loss of activity in the dark period. Much activity may also result from external stimulation, particularly from disturbances produced by laboratory personnel. Cessation of activity each day with great regularity after departure of laboratory personnel clearly establishes the importance of this source of activity. Total activity from 0600 to 1800 equals total activity present in dark before destruction of the clock.

Function of Synchronizers. In the normal rat kept under conditions of alternating 12-hr periods of light and darkness, onsets of activity and drinking and eating begin promptly at start of the dark. They are synchronized by the electric clock. Indirectly, all other functions of the body become synchronized.

The records showed that, immediately after destruction of the clock and cessation of synchronization, these functions become synchronized by the electric clock at 0600 and the time relationships of the various functions continued as before destruction of the clock.

This is a simple but remarkable instance of synchronization.

DISCUSSION

Stetson and Watson-Whitmyre (11) reported that bilateral radiofrequency lesions of the suprachiasmatic nuclei in hamsters resulted in sporadic activity in the light and dark but mainly in the light and also that the lesions abolished all cyclicity and photoperiodic photosensitivity. A larger area of the lesions or a more complete removal of all suprachiasmatic cells may account for the complete elimination of activity in the dark in the present experiments.

Moore-Ede and his coworkers (12–14) have made extensive and interesting studies on synchronizers mainly in squirrel monkeys kept in constant light.

Modern man's 24-hr clock has somehow become submerged during the process of evolution [possibly because of his discovery of fire (8)]. The clock does not manifest itself except under pathological conditions, such as brain lesions or constant light. Whether or how the various functions of the human body become synchronized remains unknown.

I acknowledge with thanks suggestions and criticisms received from Drs. Robert G. Robinson and James B. Wirth, Mr. Stephen W. Siebert, and Professors David Bodian, Charles Southwick, William R. Green, John E. Dowling, and H. David Mosier, Jr. This work was carried out under National Institute of Mental Health Grant MH00576.

1. Richter, C. P. & Wang, G. H. (1926) *J. Lab. Clin. Med.* **12,** 289–292.
2. Richter, C. P. (1965) *Biological Clocks in Medicine and Psychiatry* (Thomas, Springfield, IL).
3. Stellar, E. & Hill, J. H. (1952) *J. Comp. Physiol. Psychol.* **45,** 96.
4. Halász, B. (1969) in *Frontiers in Neuroendocrinology,* eds. Ganong, W. F. & Martini, L. (Oxford Univ. Press, New York), Vol. 1, pp. 307–342.
5. DeGroot, J. (1959) *J. Comp. Neurol.* **113,** 389–400.
6. Moore, R. Y. (1978) in *Frontiers in Neuroendocrinology,* eds. Ganong, W. F. & Martini, L. (Raven, New York), Vol. 5, pp. 185–206.
7. Richter, C. P. & Warner, C. L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 598–601.
8. Richter, C. P. (1977) *Johns Hopkins Med. J.* **141,** 47–61.
9. Richter, C. P. (1960) *Proc. Natl. Acad. Sci. USA* **46,** 1506–1529.
10. Richter, C. P. (1967) *Res. Publ., Assoc. Res. Nerv. Ment. Dis.* **45,** 8–29.
11. Stetson, M. H. & Watson-Whitmyre, M. (1976) *Science* **191,** 197–199.
12. Moore-Ede, M. C., Schmelzer, W. S., Kass, D. A. & Herd, J. A. (1977) *Am. J. Physiol.* **233,** R230–R238.
13. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1978) *Am. J. Physiol.* **234,** R130–R135.
14. Fuller, C. A., Sulzman, F. M. & Moore-Ede, M. C. (1978) *Science* **199,** 794–796.