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Circadian Rhythms in Drinking Behavior and Locomotor Activity of Rats Are Eliminated by Hypothalamic Lesions

(suprachiasmatic and medial preoptic nuclei/retino-hypothalamic projection)

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ABSTRACT **Bilateral electrolytic lesions in the suprachiasmatic nuclei permanently eliminated nocturnal and circadian rhythms in drinking behavior and locomotor activity of albino rats. The generation of 24-hr behavioral rhythms and the entrainment of these rhythms to the light-dark cycle of environmental illumination may be coordinated by neurons in the suprachiasmatic region of the rat brain. Destruction of the medial preoptic area had no effect on 24-hr drinking rhythms.**

The widespread occurrence and biological significance of 24-hr rhythms has been extensively documented (1, 2). Although rhythmic variations in animal behavior and physiology are ordinarily synchronized with fluctuations in light and temperature (1), many rhythms persist in free-running form with periods of about 24 hr (circadian rhythms) in the absence of all obvious entraining stimuli. Such demonstrations are generally interpreted as reflecting the operation of a biological clock within the animal (1).

The identification of the neural substrate responsible for the 24-hr behavioral rhythms of mammals has yet to be accomplished (3, 4). Circadian rhythms have been remarkably resistant to many forms of interference with the nervous system, including such radical treatments as anoxia, convulsions, poisoning, anesthesia, and acute stress (4). Some loss of rhythmicity in eating, but not in drinking, behavior of rats occurs after lesions are placed in the region of the ventromedial hypothalamus (5). However, these lesions also interfere with the homeostatic control of eating and body weight, and it is difficult to assess their effects on the biological clock *per se*.

Several circadian neuroendocrine rhythms have been eliminated by lesions or surgical isolation of the anterior hypothalamus from the medial basal hypothalamus (6). In addition, recent anatomical studies have once again raised the possibility of direct visual input to the anterior hypothalamus via retino-hypothalamic pathways terminating in the suprachiasmatic nuclei and arcuate region (7, 8). These considerations, and our previous failure to disrupt nocturnal drinking rhythms with lesions that interrupted the primary and accessory visual pathways (9), suggested to us that the suprachiasmatic region might be involved in the generation and entrainment of behavioral rhythms.

In the present experiment we attempted to interfere with circadian drinking and activity rhythms of rats by selectively damaging several regions of the hypothalamus.

Abbreviations: L-D, light–dark cycle; SCN, suprachiasmatic nucleus; MPO, medial preoptic nucleus.

METHODS

Adult ovariectomized Sprague–Dawley rats were housed individually in cages with free access to food and water. The experimental room was illuminated by fluorescent lights providing cool white light; the average intensity of illumination at the face of the cage was 6 ft-c. The light–dark cycle (L-D) consisted of alternating 12-hr periods of light and darkness; the dark period began at 9 p.m. At various times animals were maintained on a reversed L-D cycle (the daily 12-hr dark period beginning at 9 a.m.) or in continuous illumination. One group of rats was maintained for part of the experiment in cages that provided free access to standard activity wheels. In all experiments water intake was measured to the nearest ml with calibrated Richter tubes; intakes were recorded daily at 9 a.m. and 9 p.m. The number of revolutions traversed by animals in the running wheels was recorded on counters that were read daily at 9 a.m. and 9 p.m. An Esterline–Angus multiple channel event recorder was used to study the distribution of drinking and activity patterns of selected individual rats. Each lick at the water tube and each revolution of the activity wheel deflected a separate pen on the event recorder, which was in continuous operation over a period of many successive days; these records, when cut and assembled for individual rats, provide an index of the temporal distribution of drinking and activity.

Brain lesions were made by passage of an anodal direct current of 2 mA for 20 sec through an insect pin insulated except for 0.25 mm at the tip. Coordinates for the lesions were obtained from the Pellegrino and Cushman stereotaxic atlas (10); lesions were verified at the end of the experiment by microscopic examination of serial brain sections.

Group 1. 25 Rats received bilateral lesions aimed at the suprachiasmatic nucleus (SCN); lesion coordinates were anterior (A) 1.0, lateral (L) ± 0.3, and ventral (V) -9.2 mm.

Group 2. 20 Rats received unilateral lesions aimed at the SCN. In 10 of these animals the eye ipsilateral to the side of the lesion was enucleated; in the remaining rats the contralateral eye was removed. This procedure was followed in order to assess the possibility that only the decussated components of the visual pathways might carry information about environmental illumination to central neural pacemakers. If this were the case, removal of the eye ipsilateral to the side of the lesion could result in a free-running rhythm, despite the presence of one intact primary optic tract.

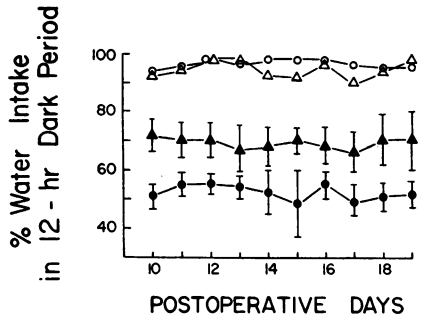

FIG. 1. Percentage of 24-hr water intake consumed by rats during the 12-hr dark period on days 10–19 after surgery. Medial preoptic lesion, O; suprachiasmatic lesion, ●; bilaterally enucleated ▲; unoperated rats, △.

Group 3. Eight rats received bilateral lesions aimed at the medial preoptic area (MPO); lesion coordinates were *A* 1.6, *L* ±0.3, and *V* −9.3 mm.

Groups 4, 5, and 6. Eight rats bilaterally blinded by orbital enucleation, five unilaterally blinded rats, and five intact animals served as controls. All groups were studied over a period of 3 months.

11 Rats in the bilateral SCN group survived after the operation; two of these developed severe polydipsia and were discarded from the experiment. 16 Rats in the unilateral SCN groups and six in the MPO group also survived the operations. These rats were tested for the presence of drinking rhythms and the ability to entrain drinking to an inverted L-D cycle. All SCN rats and five intact control rats were then studied in the activity apparatuses.

RESULTS

Water intake for 24-hr periods was stabilized at preoperative levels on no later than the seventh day after surgery, e.g., mean daily water consumption for bilateral SCN rats was 45.5 ml for the last 4 days preceding surgery and 45.9 ml for days 7–10 after operation. The percent of daily drinking occurring in the 12-hr dark period is illustrated in Fig. 1. Unoperated control rats generated striking nocturnal rhythms in water intake (see ref. 11); about 95% of their daily drinking occurred during the 12-hr dark period. Medial preoptic lesions did not interfere with nocturnal drinking rhythms (Fig. 1); these rhythms were completely eliminated in 7 of the rats in the group sustaining bilateral SCN lesions. During postoperative days 10–19, 52.2% of their drinking occurred during the dark periods*. Since rats in the SCN group also incurred extensive damage to the optic chiasm, it is necessary to demonstrate that the observed effects were not merely an artifact of blindness. During the 10th–19th postoperative days, bilaterally enucleated rats consumed about 70% of their daily water intake in the 12-hr dark periods (Fig. 1); these differences between the SCN and peripherally blinded animals were significant on each of days 10–19 ($P \leq 0.002$, Mann–Whitney U-test). Rats with SCN lesions, unlike peripherally blinded animals, permanently lost their preoperative nocturnal drinking rhythms immediately after surgery.

Six of the seven rats with effective SCN lesions showed no signs of circadian drinking rhythms over a period of 2 months. A typical drinking pattern for one of these animals is shown in the *lower portion* of Fig. 2. The drinkometer records indicated that rats with effective SCN lesions did not show free-running circadian drinking rhythms either in the L-D cycle or in constant light. This is in contrast to the free-running circadian rhythm displayed by blind rats (*top portion* of Fig. 2 and ref. 4). One of the 7 SCN rats developed a free-running circadian rhythm beginning 2.5 weeks post-operatively. The suprachiasmatic nuclei were only partially damaged in this animal, but the primary optic tracts were completely interrupted by the lesions.

The total number of revolutions recorded in the running wheels was reduced more than 50% by the SCN lesions. Nocturnal activity rhythms were completely eliminated in these animals (Table 1), and no circadian periodicity was detectable on the Esterline–Angus event records (Fig. 3, *bottom*).

Rats sustaining unilateral SCN lesions in combination with either ipsi- or contralateral enucleation initially generated attenuated nocturnal drinking rhythms of 65%; subsequently, their nocturnal drinking was stabilized at 75%. This should be compared with the 85% nocturnal drinking shown by unilaterally blinded rats. Re-entrainment of drinking after inversion of the L-D cycle was slightly slower for unilateral SCN rats than for rats sustaining only unilateral blinding. These results suggest that an uncrossed component in the visual pathway transmits information concerning the L-D cycle into the brain.

Lesions that permanently eliminated activity and drinking rhythms totally destroyed both suprachiasmatic nuclei. Adjacent structures, including the anterior hypothalamic nuclei, medial preoptic area, periventricular nuclei, arcuate nuclei, and optic chiasm were damaged to varying degrees in most animals; the presence of all or part of one SCN was sufficient to maintain at least some degree of nocturnal drinking. Ineffective lesions were either asymmetrical or

TABLE 1. *Percentage of 24-hr wheel running activity occurring during the 12-hr dark period for rats maintained on 12-hr light and 12-hr dark cycle of illumination*

Rat no.	Group*	Revolutions per 24-hr†	% of daily activity in 12-hr dark period†
226	C	811 ± 139	89 ± 6
230	C	1033 ± 199	97 ± 3
233	C	1256 ± 200	93 ± 8
4	SCN	515 ± 127	51 ± 11
9	SCN	425 ± 110	51 ± 11
18	SCN	268 ± 38	49 ± 6
28	SCN	283 ± 144	57 ± 20
228	SCN	150 ± 38	49 ± 15
234	SCN	89 ± 45	43 ± 18

* C = unoperated control rats; SCN = rats with bilateral suprachiasmatic lesions.

† Means ± SD are based on data obtained for each rat on 20 consecutive days beginning 10 days postoperatively for rats 228 and 234, and 60 days postoperatively for rats 4, 9, 18, and 28.

* The group values shown in Fig. 1 are representative of those for each of the individual animals. This is indicated by the standard deviations.

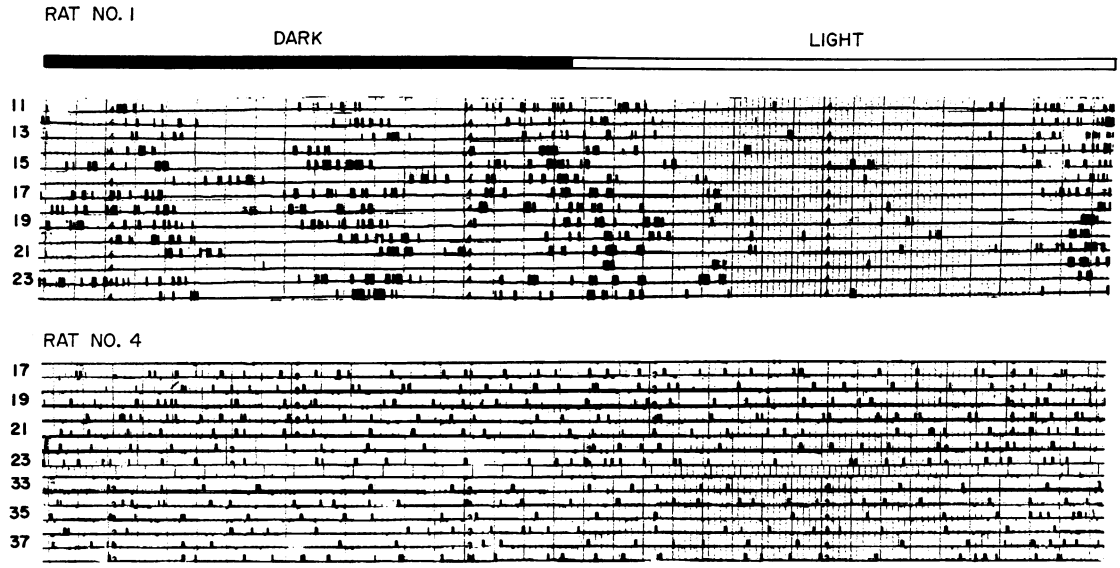

FIG. 2. (*Top*) Distribution of drinking for a blind rat (*No 1*) beginning 11 days after blinding. Note the free-running circadian rhythm. (*Bottom*) Distribution of drinking for a rat with bilateral suprachiasmatic lesions. Note the absence of nocturnal and circadian rhythmicity.

more anteriorally placed than effective ones. Fig. 4 illustrates the extent of the smallest effective SCN lesion (*Rat No. 9*). Damage to the ventro-medial hypothalamus was clearly not essential for the elimination of either drinking or activity rhythms. The greatest amount of damage to the SCN that was still consistent with a residual nocturnal rhythm in drinking is illustrated in Fig. 4 (*right*). The animal suffering this degree of neural damage (*Rat No. 16*) consistently consumed 60% of its daily water in the 12-hr dark period.

DISCUSSION AND CONCLUSIONS

The neural substrate that generates and entrains 24-hr behavioral rhythms in mammals remains largely unspecified. We have recently demonstrated that interruption beyond the chiasm of the primary and inferior accessory optic systems of the rat does not interfere with the entrainment of drinking rhythms to the illumination cycle (9). We suggested that entrainment might be accomplished via a direct retino-hypothalamic pathway (7, 8) innervating a pacemaker in the region of the suprachiasmatic nuclei. We have now demonstrated that nocturnal and free-running circadian rhythms of drinking and locomotor activity are severely disrupted by relatively discrete lesions in this region of the hypothalamus. The distribution of the drinking and activity rhythms raises the possibility that the normal sleep pattern is also modified in SCN rats. This suggestion remains to be verified by direct experimentation. The loss of drinking and activity rhythms in SCN rats occurred without any gross impairments in several

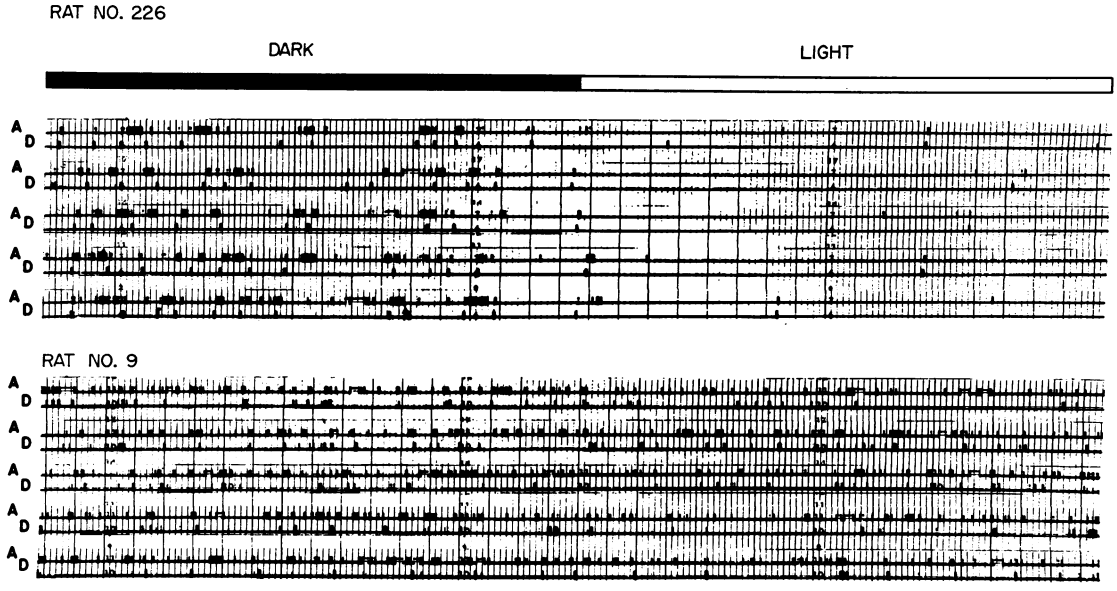

FIG. 3. Simultaneously recorded activity (*A*) and drinking (*D*) rhythms for an unoperated control rat (*No. 226, top*) and a rat with bilateral suprachiasmatic lesions (*No. 9, bottom*) during a 5-day period about 2 months after surgery. The control rat's drinking and activity are entrained to the light–dark cycle. There is no indication of entrainment or circadian periodicity in the record of the rat with the lesion.

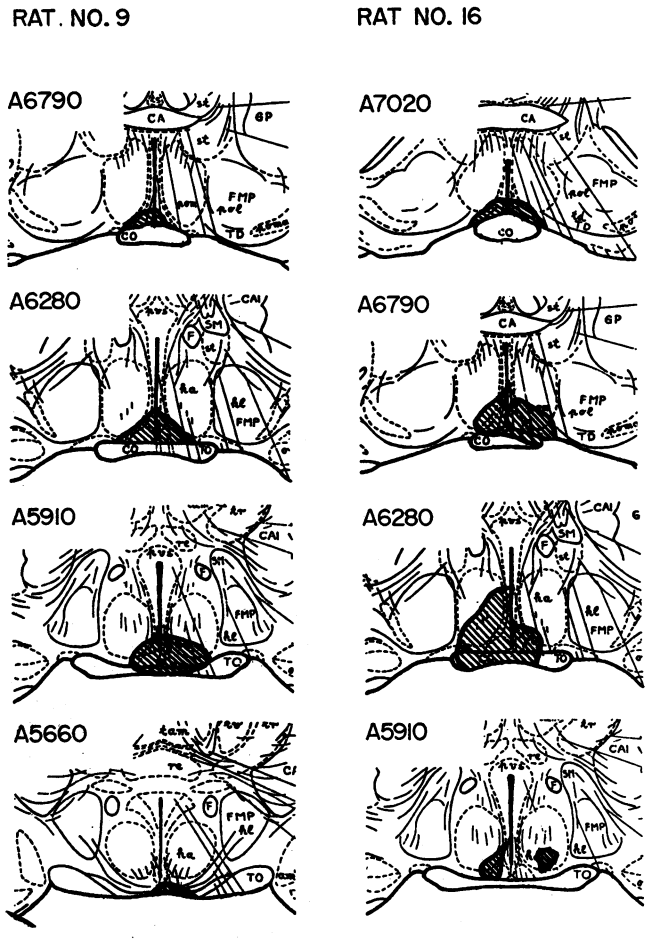

FIG. 4. (*Left*) (*Rat No. 9*) smallest suprachiasmatic lesion effective in eliminating drinking and activity rhythms. Lesion is indicated by *cross-hatched area*. (*Right*) (*Rat No. 16*) The greatest amount of damage to the suprachiasmatic nuclei that was consistent with residual nocturnal drinking rhythms. Brain sections were modified from König and Klippel (13).

homeostatic mechanisms. Thus, both water intake and body weight were maintained at normal levels throughout the 3-month period of the study. This is in contrast to previous reports of loss of eating rhythms after lesions are placed in the ventromedial hypothalamus (5)

The suprachiasmatic region of the rat brain may contain a number of pacemakers responsible for the generation of certain behavioral rhythms. Loss of the 4–5 day estrous cycle (12) as well as of other neuroendocrine rhythms (6) after formation of lesions in the suprachiasmatic-medial preoptic region implies that several rhythmic processes are dependent on the integrity of this brain area. It remains to be specified whether the neurons that generate 24-hr behavioral rhythms are themselves directly responsive to patterns of environmental illumination.

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