Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat

The role of vision in neuroendocrine regulation is well-known[1,2], but few data are available on specific visual pathways mediating such functions or their relation to brain centers controlling rhythmic events. Two sets of observations are pertinent to these problems. First, selective ablation of the inferior accessory optic tracts in the rat eliminates the response of the pineal melatonin-forming enzyme, hydroxyindole-*O*-methyltransferase (HIOMT) to light, without affecting visually guided behavioral responses, whereas section of the primary optic tracts causes a loss of visual behavior with preservation of the pineal HIOMT response to light[3–5]. Second, Critchlow[6] has found that lesions in the suprachiasmatic region of the hypothalamus abolish the constant estrous response to light in the female rat, a response that is maintained after section of the primary optic tracts. His observations are of particular interest in view of the recent demonstration of a direct retinohypothalamic projection terminating in the suprachiasmatic nuclei in the rat[7]. The purpose of the present study was to re-investigate the role of the central retinal projections in neuroendocrine regulation. In the studies noted above, only prolonged responses to continuous lighting conditions were examined. The experiments reported here were directed toward a different function, the neural regulation of a true circadian rhythm exemplified by the diurnal variation in adrenal corticosterone content[1,6].

Two experiments were performed. The subjects for each were female albino rats (Holtzman Co., Madison, Wis.) weighing approximately 180–200 g at the beginning of the experiment. Female rats were used in these experiments because their diurnal rhythm in adrenal corticosterone content has a greater amplitude than that of males[6]. They were housed in clear plastic cages (6–8 per cage) with free access to food and water. The cages were in racks illuminated by fluorescent lamps (Vita-Lite, Duro-Test Corp.) with a light emission similar to that of natural light. The animals were exposed to approximately 50 ft.-cd of illumination during the 12 h (07.00 to 19.00) the lights were on each day. All surgical procedures were performed under ether anesthesia. The animals were sacrificed on the 21st postoperative day at 4 time points around the clock, 07.00, 13.00, 19.00 and 24.00 hours. Their adrenals were removed and frozen on dry ice prior to subsequent analysis for corticosterone content using a modification of the method of Silber[8]. The location of each brain lesion was verified by histologic study.

In the first experiment 4 groups of animals were used. One was subjected to sham operation and a second to blinding by bilateral orbital enucleation. Visual pathway lesions were placed in the final two groups. In the first of these a lesion was made unilaterally to destroy the optic tract just beyond its emergence from the chiasm (stereotaxic coordinates; anterior 7 mm, lateral 1.5 mm, ventral 2.5 mm below horizontal zero with the tooth bar 5 mm above the ear bars) by passing anodal DC current of 3 mA for 60 sec through an insulated electrode. The eye ipsilateral to the lesion was then removed so that the only intact visual pathways in these animals were the retinohypothalamic projection and one uncrossed primary optic tract. Because of

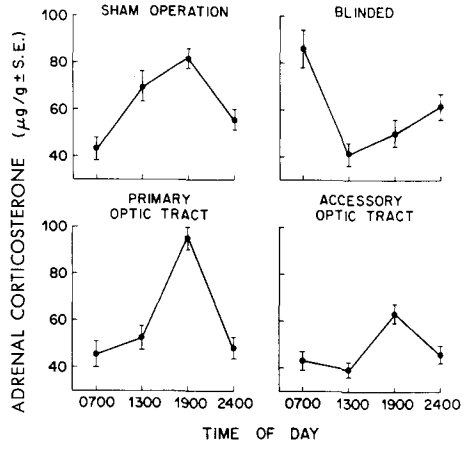

Fig. 1. Effect of visual pathway lesions on the adrenal corticosterone rhythm. Four to 6 animals were sacrificed at each time point for each operated group, 21 days after operation. The graphs demonstrate the mean values (± S.E.M.) at each time point. The 19.00 hour time point value is significantly higher than the 07.00 hour point ($P < 0.05$, t-test) for each group except the blinded group which showed a reverse rhythm with a peak at 07.00.

their complete decussation in the optic chiasm[4] the accessory optic tracts were totally transected and this group is designated the accessory optic tract group. In the second visual pathway group, primary optic tract lesions were made as described previously[3,4]. These lesions totally transected the primary optic tracts at their entry into the ventral thalamus. One inferior accessory optic tract and the retinohypothalamic projection was spared in these animals. The results of this experiment are shown in Fig. 1. The sham operated group and both visual pathway lesion groups exhibited a normal rhythm in adrenal corticosterone content with a peak value at 19.00 hours. The blinded group, in accord with Critchlow's[6] observations, showed a shift in the peak value to 07.00 hours. The peak value in the accessory optic tract group was substantially lower than that of the other groups but we have occasionally observed such variation among normal or sham operated groups. Consequently, it is difficult to interpret the low peak value in this group. The significant finding in this experiment is that neither primary nor accessory optic tract destruction alters light synchronization of the adrenal corticosterone rhythm. For this reason an additional experiment was planned to test the participation of the retinohypothalamic projection in this function.

Six groups of animals were prepared for this experiment. Sham operated and blinded groups were included as before. Bilateral electrolytic lesions were placed in the suprachiasmatic nuclei by passing anodal DC current of 2 mA for 20 sec into each nucleus (stereotaxic coordinates; anterior 7.4 mm, lateral 0.5 mm, ventral 2 mm below horizontal zero with the tooth bar 5 mm above the ear bars). Postoperatively about half of this group of 48 animals appeared to have normal vision and exhibited pupillary responses to light. In many of these animals the lesions ablated the suprachiasmatic nuclei totally (Fig. 2) but in some they spared either part or all of the suprachiasmatic nuclei and the data from the latter were excluded. The remaining animals had neither

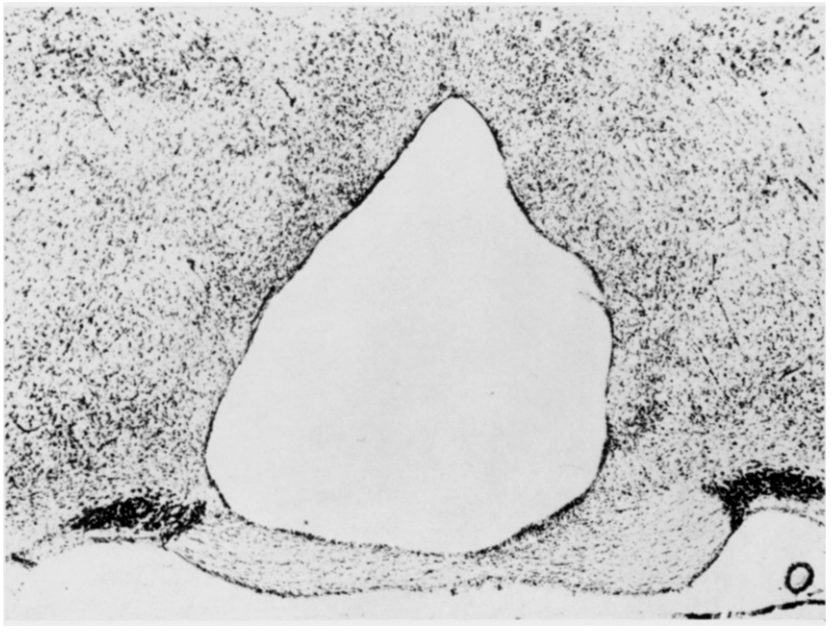

Fig. 2. Photomicrograph of a frontal section through the hypothalamus of an animal from the suprachiasmatic nucleus lesion group. The lesion ablates the hypothalamic tissue dorsal to the optic chiasm and between the supraoptic nuclei, sparing the chiasm itself. Both suprachiasmatic nuclei are totally destroyed and the lesion encroaches on adjacent nuclei of the anterior medial hypothalamus. Cresyl violet stain, × 24.

pupillary responses to light nor did they evidence any gross indication of behavioral response to visual cues and, in each of these, the lesion extended through the suprachiasmatic nuclei to transect the optic chiasm. Two additional groups were subjected to lesions and a third to a control operation using a modified Halász knife[9] which had vertical and horizontal components each 2.4 mm in length. In the first lesion group, a frontal cut across the medial hypothalamus was placed 0.5 mm rostral to the chiasm, and in the second a cut was placed 0.5 mm caudal to the chiasm. The relationship of

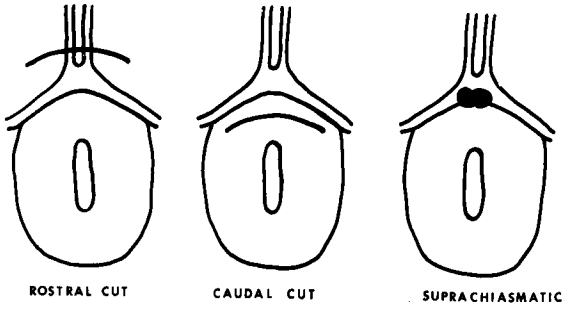

Fig. 3. Diagrammatic representation of lesion location in the second experiment. The diagrams are of the base of the rat hypothalamus showing the relative location of the lesions to the optic chiasm. The rostral frontal cut in the diagram on the left is shown as a line in front of the optic chiasm. This lesion spared the optic nerves. The caudal frontal cut is depicted in the middle figure. The location of the suprachiasmatic lesions is shown on the right.

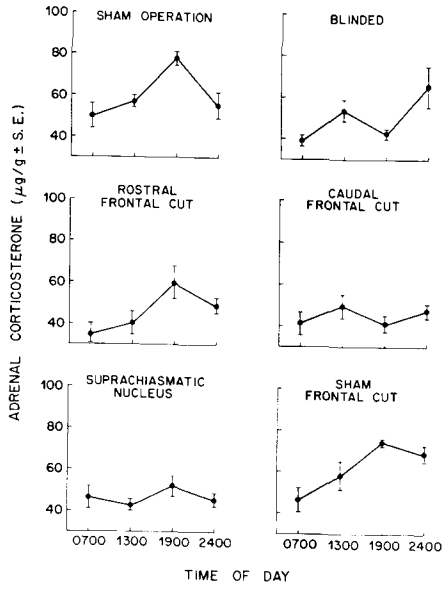

Fig. 4. Effect of hypothalamic lesions on the adrenal corticosterone rhythm. The sham operated and blinded groups each contained 4 animals per time point. The remaining groups had 6–10 animals at each point. The 19.00 hour value was significantly higher than the 07.00 value ($P < 0.05$, t-test) for the sham operated, sham frontal cut and rostral frontal cut groups indicating a normally timed, diurnal rhythm. The rhythm was shifted in the blinded group to a peak at 24.00 hours. There were no significant differences between time points for the suprachiasmatic and caudal frontal cut groups.

these cuts to each other and to the suprachiasmatic lesions is shown in Fig. 3. In the control group (sham frontal cut group) the knife was lowered to the base of the brain and then withdrawn without making a cut. The frontal cut groups were prepared in order to control for the possibility that an effect of the suprachiasmatic lesions might be due to transection of fibers passing rostrocaudally through that nucleus rather than to destruction of the nucleus itself or projections terminating in it. This possibility was brought out by Halász *et al.*[10] who found that partial deafferentation of the hypophysiotrophic area by a frontal cut caudal to the chiasm altered the P.M. rise in adrenal and blood corticosterone. In their study only two time points were sampled so that it is not clear whether the lesion abolished the rhythm or produced a shift in the peak. The results from the present study are shown in Fig. 4. Both sham operated groups exhibited a normal corticosterone peak at 19.00 hours and the blinded animals a shift in the peak value, on this occasion to 24.00 hours. This shift in peak is less than is usually observed with a 21-day postoperative survival period[6]. The rostral frontal cut animals also maintained a clear rhythm with a peak at 19.00 hours. Again, as in the accessory optic tract group of the first experiment, the peak value is somewhat lower than that of the other groups but the amplitude (about 25 μg/g) is equivalent among all of the groups that show a rhythm. The data from all animals with complete destruction of the suprachiasmatic nuclei are pooled since there were no differences between the animals with sparing or involvement of the chiasm. In this group and in the caudal

frontal cut group there was no evident rhythm, and the corticosterone levels at all time points remained in the range normally observed for morning values (Fig. 4). It is apparent from this data that the suprachiasmatic region is essential to the maintenance of a normal adrenal corticosterone rhythm in the rat. The loss of the rhythm observed in the caudal frontal cut and the suprachiasmatic groups cannot be interpreted, however, as the result of ablation of a critical visual pathway. If this were the case, a shift in the peak should have occurred as in the blinded groups. This did not occur; no peak was evident and the rhythm appeared to be abolished. It is also apparent that preservation of the primary and accessory optic tracts, as in the animals exemplified by Fig. 2, in the absence of the suprachiasmatic region, is not sufficient to maintain synchronization of the corticosterone rhythm. Since section of the primary or accessory optic tracts does not affect the adrenal corticosterone rhythm, and these tracts are unable to maintain the rhythm in the absence of the terminal nucleus of the retinohypothalamic tract, we would propose that input from the retinohypothalamic tract mediates information critical to the synchronization of that circadian rhythm. This is not conclusively demonstrated by the present data; but they do indicate that the suprachiasmatic nuclei, or closely adjacent neuronal groups, participate in the production of rhythmic events controlling the hypothalamo-pituitary regulation of adrenal function.

This work was supported by Grants NS-05002 and HD-04583 from the National Institutes of Health, U.S. Public Health Service. Victor B. Eichler was supported by a U.S. Public Health Service Postdoctoral fellowship (NS-32,771).

We are grateful to Mrs. Francis Karapas, Mrs. Aina Svanbergs and Mrs. Hamida Qavi for technical assistance.

Departments of Pediatrics,
Medicine (Neurology) and Anatomy,
and the Joseph P. Kennedy, Jr.
Mental Retardation Research Center,
University of Chicago,
Chicago, Ill. 60637 (U.S.A.)

ROBERT Y. MOORE
VICTOR B. EICHLER

1 HALBERG, F., Chronobiology, *Ann. Rev. Physiol.*, 31 (1968) 675–725.
2 WURTMAN, R. M., Effects of light and visual stimuli on endocrine function. In L. MARTINI AND W. F. GANONG (Eds.), *Neuroendocrinology Vol. 2*, Academic Press, New York, 1967, pp. 19–51.
3 MOORE, R. Y., HELLER, A., WURTMAN, R. J., AND AXELROD, J., Visual pathways mediating pineal response to environmental light, *Science*, 155 (1967) 220–223.
4 MOORE, R. Y., HELLER, A., BHATNAGAR, R. K., WURTMAN, R. J., AND AXELROD, J., Central control of the pineal gland: Visual pathways, *Arch. Neurol. (Chic.)*, 18 (1968) 208–218.
5 CHASE, P. A., SEIDEN, L. S., AND MOORE, R. Y., Behavioral and neuroendocrine responses to light mediated by separate visual pathways in the rat, *Physiol. Behav.*, 4 (1969) 949–952.
6 CRITCHLOW, V., The role of light in the neuroendocrine system. In A. NALBANOV (Ed.), *Advances in Neuroendocrinology*, Univ. of Illinois Press, Urbana, Ill., 1963, pp. 377–401.
7 MOORE, R. Y., AND LENN, N. J., A retinohypothalamic projection in the rat, *J. comp. Neurol.*, in press.
8 SILBER, R. H., Fluorimetric analysis of corticoids. In D. GLICK (Ed.), *Methods of Biochemical Analysis, Vol. 14*, Interscience, New York, 1966, pp. 63–78.
9 HALÁSZ, B., AND PUPP, L., Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area, *Endocrinology*, 77 (1965) 553–562.

10 HALÁSZ, B., SLUSHER, M. A., AND GORSKI, R., Adrenocorticotrophic hormone secretion in rats after partial or total deafferentation of the medial basal hypothalamus, *Neuroendocrinology*, 2 (1967) 43–55.

(Accepted April 7th, 1972)