PHOTIC AND NEURAL CONTROL OF THE 24-HOUR NOREPINEPHRINE RHYTHM IN THE RAT PINEAL GLAND

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ABSTRACT

WURTMAN, RICHARD J., JULIUS AXELROD, GÖRAN SEDEVALL AND ROBERT Y. MOORE: Photic and neural control of the 24-hour noepinephrine rhythm in the rat pineal gland. J. Pharmacol. Exp. Therap. 157: 487–492, 1967. The noepinephrine content of the rat pineal gland varies 3-fold during each 24-hr day. Noepinephrine levels are highest at the end of the dark period and fall during the light period. This rhythmic variation in pineal noepinephrine is abolished when animals are blinded or are kept in continuous light or darkness. It appears to be generated by nerve impulses which are initiated by photic stimulation of the retina. These impulses are carried to the brain by the inferior accessory optic tract and reach the pineal by a pathway which includes the preganglionic sympathetic trunk to the superior cervical ganglion.

The rat pineal gland is unusually rich in such biogenic amines as serotonin (Quay, 1963), histamine (Machado et al., 1965), dopamine and noepinephrine (Pellegrino de Iraldi and Zieher, 1966), as well as in enzymes which synthesize and metabolize these compounds (Wurtman et al., 1963; Lovenberg et al., 1967; McGeer and McGeer, 1966; Snyder et al., 1965a). In this species, pineal serotonin is stored in both the parenchymal cells and the sympathetic nerve endings (Bertler et al., 1963), while the noepinephrine is highly localized to the nerve endings (Pellegrino de Iraldi and Zieher, 1966). Pineal sympathetic denervation (by bilateral destruction of the superior cervical ganglia) has been shown to deplete the gland of almost all of its noepinephrine (Pellegrino de Iraldi and Zieher, 1966).

The amounts of serotonin present in the rat (Quay, 1963) and monkey (Quay, 1966) pineal gland are not constant, but vary in a predictable manner during each 24-hr day. Serotonin content is highest in the middle of the daylight period, and falls with the onset of darkness (Quay, 1963). The activity of the pineal enzyme, hydroxy-indole-O-methyl transferase (HIOMT), which converts N-acetylseryotonin to melatonin, also varies with a 24-hr period. It is lowest at the end of the day and rises with darkness (Axelrod et al., 1965). Both of these rhythms are controlled by the sympathetic nerves to the pineal gland (Axelrod et al., 1965; Fiske, 1964). This report describes a 24-hr rhythm in the noepinephrine content of the pineal gland and defines some neural pathways responsible for its control.

METHODS. Adult female Sprague-Dawley rats weighing 160 to 200 g were used in all experiments. The rats were maintained under controlled lighting conditions for at least 1 week prior to use. Light was provided by cool white
fluorescent lamps; each animal was exposed to about 25 to 50 footcandles of illumination. In “diurnal lighting” experiments lights were on from 7 A.M. to 7 P.M. Animals were blinded by bilateral orbital enucleation (performed under light ether anesthesia), or the preganglionic fibers to their superior cervical ganglia were transected as described before (Snyder et al., 1965b). Bilateral transection of the inferior accessory optic tract was performed by unilateral enucleation and ipsilateral section of the medial forebrain bundle (Moore et al., 1967); the location of each medial forebrain bundle lesion was identified by histologic examination.

The norepinephrine content of the pineal gland at any particular time was determined using glands from groups of 12 to 20 rats. Two or three organs were pooled for each assay. The pineals were homogenized in 5 ml of perchloric acid (0.4 N). After centrifugation, the supernatant was transferred to a 25-ml beaker containing 200 mg of acid-washed alumina, 5 ml of 2 N EDTA and 15 mg of sodium bisulphite. The pH was adjusted to 8.6 with concentrated solutions of potassium hydroxide, and the contents were poured over a column containing an additional 200 mg of alumina which had been brought to pH 8.6. The column was then washed with 0.2 N sodium acetate and glass-distilled water, and the norepinephrine was eluted with three 1-ml portions of 0.2 N acetic acid. Using this procedure, the recovery of catecholamine averaged 70 ± 2%.

The norepinephrine was assayed spectrophotofluorometrically (Euler and Lishajko, 1961).

RESULTS. Twenty-four-hour variation in pineal norepinephrine content and effect of blinding. Previous experiments had shown that the content of norepinephrine in the rat pineal gland varies markedly during a 24-hr period (Wurtman and Axelrod, 1966). When rats were kept under alternating 12-hr periods of light and darkness, pineal norepinephrine levels were greatest at the end of the dark period and fell continuously during the light period (fig. 1).

To examine the influence of environmental lighting on the rhythms, rats were blinded by bilateral orbital enucleation and kept with sham-operated animals under diurnal lighting conditions for 5 days. They were then killed, and their pineals were analyzed for norepinephrine content. In contrast to control animals, blinded rats showed no alteration in pineal norepinephrine levels during the 24-hr day (fig. 1). At each time point studied, the pineal norepinephrine content in the blinded animals was approximately intermediate between the maximum and minimum values observed in sham-operated rats.

Effect of continuous light or darkness on pineal norepinephrine rhythm. The abolition of the 24-hr changes in pineal norepinephrine levels by blinding suggested that this rhythm was generated by diurnal changes in environmental lighting, perceived by the retinas. If the rhythm were dependent upon cyclic lighting cues, it might be expected to disappear in rats deprived of such cues by maintenance under constant lighting conditions.

Groups of rats were kept under diurnal
lighting or continuous light or darkness for 7 days. Animals were killed at times corresponding to the expected peak and trough in pineal norepinephrine levels (7 a.m. and 7 p.m.), and their glands were assayed for the catecholamine. Among rats kept under diurnal lighting, the pineal norepinephrine content at 7 a.m. was 3 times higher than at 7 p.m. However, the norepinephrine content of glands taken from rats kept in either continuous light or darkness was low and was similar at both times of day (fig. 2).

In another experiment the dependence of the norepinephrine rhythm on light was examined by exposing animals to a reversed lighting schedule (lights on from 7 p.m. to 7 a.m.) for 8 days. The rats were then killed at 4 p.m. (after 9 hr of darkness), and their pineal norepinephrine content was compared with that of control animals killed at 5 p.m. (after 10 hr of light). Although both groups of rats were killed at the same time of day, animals that were then in darkness had about 3 times as much norepinephrine in their pineal glands as rats which were then in normal diurnal lighting (7.10 ± 0.65 vs. 2.33 ± 0.35 ng/gland; mean ± S.E.).

Neural pathway controlling the norepinephrine rhythm in the pineal. It had previously been shown that biochemical rhythms in the pineal gland were abolished following its sympathetic denervation (Axelrod et al., 1965; Fuxe, 1964; Snyder et al., 1965b). Because most or all of the norepinephrine in this organ is present within sympathetic nerve endings,

![Fig. 2. Pineal norepinephrine levels in continuous light or darkness. Rats were kept under diurnal lighting or continuous light or darkness for 7 days.](image)

![Fig. 3. Pineal norepinephrine levels following preganglionic decentralization of the superior cervical ganglia. Animals were kept under diurnal lighting for 14 days (experiment I) or 21 days (experiment II).](image)

it was not feasible to study the effect of ganglionectomy on the rhythmic changes in pineal norepinephrine levels. Instead, the flow of impulses from the central nervous system to the pineal sympathetic nerves was interrupted by cutting the preganglionic fibers to the superior cervical ganglia. Rats prepared in this fashion and control animals were kept under diurnal lighting for 2 weeks; they were then killed at 8 a.m. or 5 p.m., and their pineals were assayed for norepinephrine. The expected daytime fall in norepinephrine content was observed in pineals from control animals. However, the catecholamine level in the decentralized organs was very low, and was similar at both times studied (fig. 3, part I).

It was possible that the norepinephrine rhythm has persisted in the decentralized gland, but that its period was no longer exactly 24 hr. The times of day corresponding to maximum and minimum norepinephrine levels might then have shifted, thereby obscuring the rhythm. For this reason, another experiment was performed in which rats kept under diurnal lighting were killed at four times of day, 3 weeks after surgery. Again, norepinephrine levels were low and approximately equal in the decentralized rats at all times studied (fig. 3, part II), whereas normal animals showed the usual diurnal variation at 8 a.m. and 5 p.m. These observations indicated that the nervous pathway by which light impulses produce the norepinephrine rhythm includes the brain and the sympathetic nerves to the pineal.

To study the central nervous pathways which
Four weeks after surgery, all groups were placed under diurnal lighting for 14 days; they were then killed at various times. As expected, the norepinephrine rhythm persisted in animals without one eye, but was abolished following bilateral enucleation. Rats subjected to section of the inferior accessory optic tracts also failed to show a 24-hr variation in their pineal norepinephrine levels (fig. 4).

**Discussion.** Many biologic functions have been shown to vary with a 24-hr period (Aschoff, 1965). Most of these rhythms appear to be endogenous. They persist when an animal is deprived of external lighting cues (i.e., after being blinded or being kept under continuous darkness). Under these conditions, the rhythmic functions can sometimes be shown to be "free-run"; i.e., their period changes from exactly 24 hr to something more or less depending upon the species (Aschoff, 1960). Such rhythms have been called "circadian" (Halberg, 1959).

The mammalian pineal gland appears to be unusual in that at least two of its biochemical functions vary with a 24-hr rhythm which is not endogenous but is generated by the external light input. The daily rise and fall in HIOMT activity can be abolished if rats are blinded or are maintained in darkness (Axelrod, 1965). The studies described in this report indicate that a similar exogenous mechanism generates the 24-hr rhythm in pineal norepinephrine content. Evidence for this hypothesis may be summarized as follows: 1) the rhythm is lost in blind animals, even though they are maintained under diurnal lighting (fig. 1); 2) pineal norepinephrine levels are the same at 7 a.m. and at 7 p.m. in rats kept in continuous darkness (fig. 2); 3) cutting the inferior accessory optic tract abolishes the norepinephrine rhythm in the pineal even though the animals are kept under diurnal lighting and can perceive visual cues through the remaining eye and the primary optic tracts (fig. 4). The experiments described here indicate that the photic information which generates the norepinephrine rhythm reaches the rat pineal gland via the following route. Light impinges upon the retina, generating nerve impulses. These travel via the inferior accessory optic tract to its terminal nucleus in the rostral midbrain.
tegmentum (Hayhow et al., 1960; Moore et al.,
1967). From this point they are carried via
an unknown pathway through the brainstem
and spinal cord to the neurons of the inter-
mediolateral cell column, which supply the
preganglionic innervation of the superior
cervical ganglia. They then traverse the post-
ganglionic sympathetic axons of the nervi conarii
to the parenchymal cells of the pineal gland.

Several possible mechanisms could operate
at the cellular level to produce the 24-hr
rhythm in pineal norepinephrine content.
First, more norepinephrine could be syn-
thetized at night. This is suggested by the
observation that tyrosine hydroxylase activity
in the rat pineal varies over a 24-hr period
in the same manner as norepinephrine con-
tent (McGeer and McGeer, 1966). Since this
enzyme is thought to control the rate of
norepinephrine synthesis in vivo (Levitt et al.,
1965), increased tyrosine hydroxylase activity
may represent a basis for increased norepi-

nephrine synthesis. Second, less norepinephrine
might be released from the pineal sympa-
thetic nerves at night while synthesis con-
tinued at a steady rate. A similar mechanism
has been suggested as the basis for the rhythm
in pineal serotonin content (Snyder and
Axelrod, 1965). Third, more norepinephrine
might be metabolized in situ by monoamine
oxidase or catechol-O-methyl transferase
during the daytime. There is no evidence to
support this, however, and in vitro studies
have failed to demonstrate any changes in
monoamine oxidase activity during the 24-hr
day (Snyder and Axelrod, 1965).

The synthesis of norepinephrine in sympa-
thetic nerve endings appears to depend
upon the rate at which impulses reach
the nerve endings. Ganglionic decentralization
depresses the rate at which isotope-labeled
tyrosine is converted to norepinephrine
in the rat salivary gland (Musacchio and
Weise, 1965); ganglionic stimulation has an
opposite effect (Sedvall and Kopin, 1967).
Similarly, pineal norepinephrine levels fall
during decentralization of the superior
cervical ganglion (fig. 3; Pellegrino de Iraldi
and Zieher, 1966). Thus, it is possible that
the nocturnal increase in pineal norepineph-
rine levels results from enhanced sympathetic
nervous activity, perhaps in response to dark-
ness. Further evaluation of this hypothesis
would require direct monitoring of sympa-
thetic electrical activity under conditions of
light and darkness in nocturnal species.

The observations reported here are consist-
ent with the hypothesis that a cyclic varia-
tion in the release of norepinephrine from
sympathetic nerve endings controls some or all
of the other 24-hr rhythms in pineal biochemi-

cal function. A similar rhythm in norepi-

nephrine content has been demonstrated in the
salivary gland (Wurtman and Axelrod, 1966).
It would seem possible that such cyclic varia-
tions in tissue norepinephrine content and
release might result in parallel alterations in
the physiologic responses to drugs which act
by releasing catecholamines.

REFERENCES

Aschoff, J.: Exogenous and endogenous com-
ponents in circadian rhythms. Cold Spring
Aschoff, J.: Circadian Clocks, North Holland,
Amsterdam, 1965.
Axelrod, J., Snyder, S. H., Heller, A. and Moore,
R. Y.: Light induced changes in pineal hydroxy-
indole-O-methyltransferase: Abolition by lateral
hypothalamic lesions. Science (N.Y.) 154: 898-
899, 1966.
Axelrod, J., Wurtman, R. J. and Snyder, S. H.: Con-
tro1 of hydroxyindole-O-methyltransferase
activity in the rat pineal gland by environmental
Bentley, A., Falck, B. and Owman, C.: Cellular
localization of 5-hydroxytryptamine stores in
Euler, U. S., von Lishajko, F.: Improved

technique for the fluorimetric estimation of
catecholamines. Acta Physiol. Scand. 51:
Fiske, V. M.: Serotonin rhythm in the pineal
organ: Control by the sympathetic nervous sys-
Halberg, F.: Physiologic 24-hour periodicity;
general and procedural considerations with refer-
ence to the adrenal cycle. Z. Vitamin- Hormon-
Hayhow, W. R., Webb, C. and Jarvis, A.: The ac-
cessory optic fiber system in the rat brain. J.
Levitt, M., Spector, S., Sjoerdinga, A. and Uden-
friend, S.: Elucidation of the rate-limiting step
in norepinephrine biosynthesis in the perfused
Lovenberg, W., Jezquier, E. and Sjoerdinga, A.: Tyr-
ptophan hydroxylation: Measurement in pina-

el gland, brainstem and carcinoid tumor. Science
Machado, A. B. M., Faleiro, L. C. M. and Da
Silva, W. D.: Study of mast cell and histamine
contents of the pineal body. Z. Zellforsch. Mikro-
Musacchio, J. M. and Weise, V. K.: Effects of
decentralization on norepinephrine biosynthesis