slices were rinsed with fresh medium and measured for radioactivity or transferred to small (250 µl) agitated glass cups, one slice into each, to be superfused with Krebs-Ringer-Hepes medium at a rate of 0.25 ml/min. Both incubation and superfusion were carried out under pure oxygen at 310 K in a shaking water bath. The superfusions were commenced with plain Krebs-Ringer-Hepes medium up to the first 30 min. The period from 20 to 30 min served as period for 'basal' efflux of each individual slice. Stimuli with modified media were applied for 10 min from 30 to 40 min. The superfusates were collected in 2-min (0.5 ml) fractions for radioactivity determination. After the superfusions the slices were weighed, homogenized in 5% (w/v) trichloracetic acid solution and centrifuged, and the resulting clear supernatants measured for radioactivity by liquid scintillation counting. The efflux rate constants for various experimental phases were computed as negative slopes for the regression lines of the logarithm of radioactivity percentage remaining in the slices versus superfusion time[10].

Neither the uptake of noradrenaline nor that of taurine was markedly affected by tetanus toxin. The initial noradrenaline uptake increased slightly when tetanus toxin was present extracellularly but not after the internalization of the toxin, i.e. during the last 10 min of incubation (table 1). In contrast, taurine uptake was only then reduced by the toxin (table 2). Both taurine[11] and the toxin[12] are subject to vinblastine-sensitive fast axonal transport and thus the toxin may interfere with taurine transport at the level of the microtubular system of nerve endings. The long-term accumulation of noradrenaline was influenced by the toxin, nor was that of taurine.

Noradrenaline, a catecholamine, is stored in synaptic vesicles in the brain with a tissue concentration in the micromolar range[13], while taurine, an amino acid, is present at millimolar concentrations with only a fraction sequestered to synaptic vesicles[8]. In spite of these differences, the effects of tetanus toxin on the release of both compounds elicited by various chemical stimuli exhibited a similar pattern; only the ouabain-evoked release was enhanced by the toxin (tables 3 and 4). The responses to the two other stimuli, K^+ ions and veratrine, which depolarize the cells and induce influx of Na^+ ions, and the response to the omission of sodium ions, were not modified by tetanus toxin.

The evoked release of noradrenaline was strictly dependent on Ca^{2+} ions with all types of stimuli (table 3). The basal efflux of taurine was increased by the absence of Ca^{2+} ions from the superfusion medium (table 4). However, the evoked release of taurine was only slightly influenced by the omission of Ca^{2+}, except in the case of ouabain stimulation. Then tetanus toxin also caused an enhancement which was of about the same magnitude as with noradrenaline. The effect of the toxin on the ouabain-evoked release thus manifests itself only when in the presence (noradrenaline) or in the absence (taurine) of calcium the efflux was significantly increased over the basal levels. The concentrations of ouabain required to elicit a stimulation had to be 10 times as great with noradrenaline as the concentration effective with taurine.

Our previous studies have shown that a slight enhancement similar to the above also occurs in the ouabain-evoked GABA release[3]. The intracellular presence of tetanus toxin thus seems to cause a general increase in neurotransmitter release when Na^+, K^+-ATPase is inhibited by ouabain. The gangliosides G_{M1}, G_{D1b} and G_{T1b} increase the activity of Na^+, K^+-ATPase[14] and tetanus toxin has a high affinity for the two last-mentioned gangliosides[15]. However, we prefer not to conclude that there is a direct interaction with the toxin and Na^+, K^+-ATPase, since there is no apparent reason why the basal unstimulated release of neurotransmitters would not also be enhanced by the toxin, and this was nevertheless not the case.

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Central neural control of pineal melatonin synthesis in the rat[1]

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Summary. To investigate a possible central neural influence on nocturnal pineal metabolic activity in rats, frontal transsections of the stria medullaris thalami were conducted. Enzymes involved in melatonin synthesis, i.e. N-acetyltransferase and hydroxyindole-O-methyl-transferase, exhibited reduced activities in operated animals when compared to controls. These results indicate a modulatory role of central structures on nocturnal pineal indole metabolism.
Key words. Central innervation; melatonin synthesis; pineal gland; rat.

During the last decade, a number of morphological[3] and electrophysiological[4–6] investigations have provided compelling evidence that nerve fibers located in diencephalic and mesencephalic areas invade the deep pineal gland and extend into the pineal stalk and the superficial pineal gland. In the rat, recent neuronal tracing studies[7] have shown that fibers, probably originating in the hypothalamic paraventricular nucleus (PVN) and the septal area, project directly to the pineal via the stria medullaris thalami.

Although the peripheral sympathetic innervation originating in the superior cervical ganglia is known to exert the predominant control over pineal function[8], it appeared worthwhile to study which role the central innervation plays in regulating nocturnal pineal melatonin synthesis. In the present investigation we determined the nocturnal N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT) activities as well as melatonin content in the rat pineal following transection of forebrain structures.

Materials and methods. Experiments were performed in spring on 46 adult male Sprague-Dawley rats (b.wt 200–230 g) housed for at least one week under a 12:12 h photoperiod (lights on at 07.00 h) with food and water ad libitum. On the day of experimentation, animals underwent tribromethanol anesthesia (0.3 g/kg b.wt) between 11.00 and 13.00 h.

For the first experiment, 23 rats were divided into 4 groups of 4–7 animals each. Animals of group 1 were anesthetized only and served as controls. Group 2 animals were sham-operated and received only transection of the sagittal sinus as control for circulatory disturbances following surgery. For brain lesionings, rats of groups 3 and 4 were fixed in a stereotaxic apparatus. A transection of the stria medullaris thalami was carried out on the animals of group 3 by lowering a small surgical knife 6 mm below the cortical surface (3 mm caudal to the bregma) and moving it laterally 2.5 mm in both directions (fig. 1), according to a stereotaxic atlas[9]. This procedure transects the stria medullaris thalami and the superiorly located neocortical areas 4 and 29 and the hippocampal regions CA3 and CA4. Animals of group 4 received the same transection but the stria medullaris was left intact. This was accomplished by lowering the knife not more than 3 mm (only neo- and allocortical transections). After surgery, the excised piece of skull was returned into place and the scalp was closed with wound clips. Animals were allowed to recover from anesthesia and were immediately returned to their cages and exposed to the original photoperiod.

During the night following surgery, animals of each group were lightly etherized and sacrificed under dim red light at 03.00 h. Individual pineal glands were quickly removed and frozen in liquid nitrogen. Brains from operated animals were fixed in formalin for later verification of the extent of transection. Within 8 h following sacrifice, pineal NAT (EC 2.3.1.5) and HIOMT (EC 2.1.1.4) activities were assayed by radioenzymatic methods[10,11].

In a second experimental series, 23 rats (4 groups of 4–8 animals each) were treated in a manner identical to the first experiment but were sacrificed at 06.30 h, i.e. 30 min before light onset. In this experiment, frozen aliquots of the pineal homogenates were additionally assayed for melatonin by means of radioimmunoassay[12], with an intraassay coefficient of variation of 4.2% (for 100 pg; n = 6) and a sensitivity of 5 pg/tube.

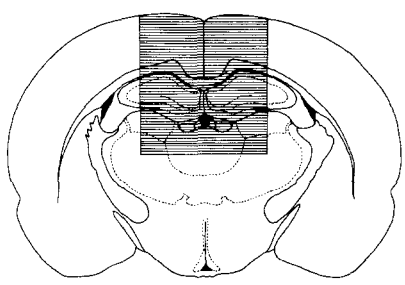

Figure 1. Drawing of a frontal section through the rat brain at the rostral end of the habenular nuclei. The shaded area corresponds to the extent of the lesion in the animals with a complete epithalamic lesion.

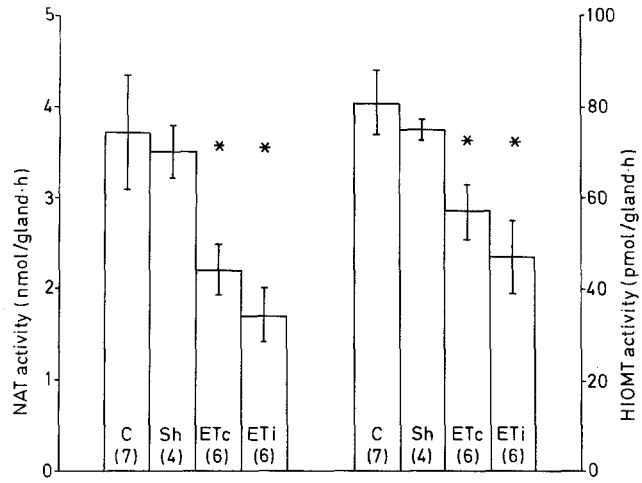

Figure 2. The effects of epithalamic transections on pineal enzyme activities at 03.00 h (4 h before light on). NAT (left) and HIOMT (right) activities are expressed as mean values ± SEM. Asterisks indicate a statistically significant difference ($p < 0.05$) relative to control values. C, Control animals; Sh, Sham-operated animals; ETc, animals receiving a complete epithalamic transection; ETi, animals receiving an incomplete epithalamic transection.

Results were statistically assessed by means of an analysis of variance followed by Duncan's multiple range comparison of means.

Results. The effects of forebrain transection on pineal enzyme activities at 03.00 h (4 h before light onset) are shown in figure 2. Sham-operated animals (group 2) exhibited enzyme activities equivalent to control levels (group 1). With respect to both control groups, animals with stria medullaris transection (group 3) and animals with cuts excluding the stria medullaris (only comprising the neocortex and hippocampus; group 4 showed significantly ($p < 0.05$) reduced pineal NAT and HIOMT activities. Mean values were reduced by roughly 50% (NAT) and 35% (NIOMT), respectively.

In animals sacrificed at 06.30 h (30 min before light onset), enzyme activities and melatonin content of control animals were found to be low (table), in line with the known fact that melatonin synthesis decreases in the morning. Pineals of animals receiving only a sagittal sinus transection (group 2) exhibited nearly identical values compared to controls (group 1). In operated animals of groups 3 and 4, mean values of both NAT and HIOMT activities were augmented by roughly 25% and 10%, respectively, compared to those found in rats of groups 1 and 2, but without reaching statistical significance. Pineal melatonin content was augmented by more than 100%, without reaching statistical significance, in rats receiving stria medullaris transections (group 4). In animals with neo- and allocortical transections (group 3), no changes were apparent.

Discussion. It is well established that pineal melatonin synthesis in rats is activated at night through the increased release of norepinephrine from sympathetic postganglionic fibers of the superior cervical ganglia[13]. The initiating signal for this nocturnal increase comes from the hypothalamic suprachiasmatic nuclei[14]. A recently discovered component of the suprachiasmatic-pineal pathway is the hypothalamic paraventricular nucleus (PVN)[15,16], which projects not only caudally to the spinal cord[17] but also dorsally to the epithalamus, including the pineal gland[18].

The effects of epithalamic transection (ET) on pineal biochemistry at 06.30 h (30 min before light onset)

Treatment		NAT pmol/organ·h	HIOMT pmol/organ·h	Melatonin pg/organ
C	(n = 5)	481.1 ± 74.6	61.3 ± 6.2	362.3 ± 138.8
Sh	(n = 4)	460.1 ± 82.4	60.1 ± 5.3	370.2 ± 82.1
ETc	(n = 8)	601.2 ± 70.1	69.5 ± 5.1	873.5 ± 325.4
ETi	(n = 6)	621.1 ± 48.7	68.4 ± 6.4	400.6 ± 21.0

Results are expressed as means ± SD. C, Control animals; Sh, Sham-operated animals; ETc, Animals receiving a complete epithalamic transection; ETi, Animals receiving an incomplete epithalamic transection.

In a recent anterograde tracing study[7], projections from the PVN to the pineal have been demonstrated to be located in the stria medullaris thalami. This observation inspired the present study in which the striae medullaris were cut bilaterally to examine what effects this might have on pineal melatonin synthesis. The results obtained show a clear decrease of NAT and HIOMT activities at night (= 3.00 h). These effects were not merely the consequence of a disturbed blood flow in the vicinity of the pineal, since no changes in pineal function were noted in sham-operated animals which had received only sagittal sinus-transection. However, in groups with lesions of the hippocampus and certain neocortical areas only, a decrease in the activity of both enzymes was also observed which suggests that the hippocampal formation itself or other efferent fibers influence pineal biochemistry. As shown by electrophysiology, the hippocampus is multisynaptically connected to the pineal gland[5]. Furthermore, hippocampal projections via septum and stria medullaris thalami terminate in the habenular nuclei[19] which in turn project to the pineal gland.

The role of the hypothalamus for pineal function has been investigated previously. HIOMT regulation was abolished following lateral hypothalamic lesions[20]. The circadian rhythmicity of pineal melatonin synthesis was disrupted by lesions of the hypothalamic paraventricular area[15] and electrical stimulation of the PVN resulted in a decrease of pineal NAT activity and melatonin content[16].

Whether the reduction in pineal NAT and HIOMT following epithalamic transection is the result of an overall attenuation of pineal metabolic activity or of a phase shift of the melatonin synthesis rhythm, cannot yet be resolved. The observation that pineal enzymes and melatonin content in operated animals remained elevated at the end of the dark period (table) speaks in favor of a phase shift of the circadian rhythm of melatonin synthesis.

That epithalamic inputs do influence the pineal's capacity to synthesize melatonin is an important new insight into the nature of brain-pineal interrelationships. On the basis of electron microscopic studies in the rat pineal[21], in which it was reported that axo-axonic synapses are occasionally present, it may be speculated that epithalamic neurons may serve to amplify in a tonic fashion the sympathetic neural activity in the pineal, or alternatively, that epithalamic neurons may serve a synergistic role in the control of melatonin synthesis by pinealocytes. In either case, without the presence of epithalamic inputs, pineal biochemical activity is altered. Parenthetically, it is interesting to note that lesions of the stria medullaris result in a decline of choline-acetyltransferase activity in the rat habenular nuclei[19] which are known to project afferent fibers to the pineal gland[22].

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Immunoreactive luteinizing hormone-containing neurons in the brain of the white-footed mouse, *Peromyscus leucopus*

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Summary. The distribution of immunoreactive LH in the brain of the white-footed mouse *(Peromyscus leucopus)* was determined using immunocytochemical procedures. Immunoreactive fibers are located in the hypothalamus, preoptic area, septum and amygdala. Stained cell bodies are seen in the arcuate nucleus and preoptic area. Gonadectomy enhances staining for LH in the brain.
Key words. Luteinizing hormone; immunocytochemistry; brain; white-footed mouse.

Anterior pituitary-like gonadotrophic hormones have been detected in the brain by means of radioimmunoassay and immunocytochemistry[2]. The presence of luteinizing hormone (LH) in the hypothalamus, limbic and striatal regions of rat brain[2-5] is thought to underlie a 'short' loop feedback mechanism whereby the hormone regulates its own secretion[6]. Changes in firing of