phologically on the basis of previous studies in which the cells were obtained directly from the surface of opened vessels under histological control and counted by the same method[2].

Albino female rats (Wistar), at the age of 3 months and weighing 180–220 g, were used in the present experiments. Nicotine was administered i.v. in the volume of 1 ml/200 g dissolved in physiological saline or orally 2 ml/200 g as aqueous solution by a gastric tube. Citrated blood was obtained from hearts of anaesthetized animals (sodium thiopental 20 mg/kg b.wt) for cell counting using siliconized collecting material. The time intervals between nicotine administration and blood collection were 5 min with the i.v. injection and 2 h with the oral administration.

Figure 1 shows a highly significant increase of cell counts after nicotine administered either i.v. or p.o. in a fraction of the dose corresponding to that absorbed during smoking of 1 cigarette (0.05 mg/kg b.wt). The statistical significances were calculated by the t-test, as in previous studies with larger groups of animals, the endothelaemic response to various stimuli has shown normal distribution. Similar repeated stimuli for detachement of cells produced serious changes in permeability of vessel walls resulting eventually in degenerative changes.

Several drugs have been shown to possess a protective effect on endothelium against various injurious agents. An outstanding position among them is occupied by flavonoids, particularly by their newer semisynthetic derivatives[3]. Consequently, 2 such derivatives were investigated for the ability to prevent increases of endothelial counts after nicotine. The lowest fully effective dose of i.v. administered nicotine (0.0125 mg/kg) was selected as a standard injury. The flavonoids, mono-7-hydroxyethylrutoside (mono-7-HR) and a mixture of hydroxyethylated rutosides (HR), were administered in various doses orally 2 h before the i.v. nicotine administration. Figure 2 shows that, even at a very low dose level representing one-twentieth of the clinical single dose, both drugs completely blocked the endothelaemia increase after nicotine.

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The effects of dopamine, noradrenaline and serotonin in the visual cortex of the cat

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Summary. The predominant effect of dopamine, norepinephrine and serotonin on the photically-evoked unitary activity was a prolonged inhibition of firing. These amines were also able to block acetylcholine-induced excitations and for longer periods of time than GABA.

Evidence supporting specific afferent pathways containing the biogenic amines dopamine (DA)[2-4], norepinephrine (NE)[5,6] and serotonin (5-HT)[7,8] has been reported in the mammalian CNS. These studies include histofluorescent microscopy, biochemical studies on the release of the amines[9] and radioautography at the light[10,11] and EM[12,14] levels, and would imply that the biogenic amines act as specific and classic neurotransmitters. However, the fact that such terminals are relatively few in number and lack specialized and specific synaptic contacts[12,13] suggests a 'modulatory' rather than a true neurotransmitter role[12-16]. In an attempt to determine the functional role of the biogenic amines in cerebral cortex we examined their effects upon cortical neurons in the visual (occipital) cortex and their interactions with the neurotransmitters acetylcholine (ACh) and γ-amino-n-butyric acid (GABA).

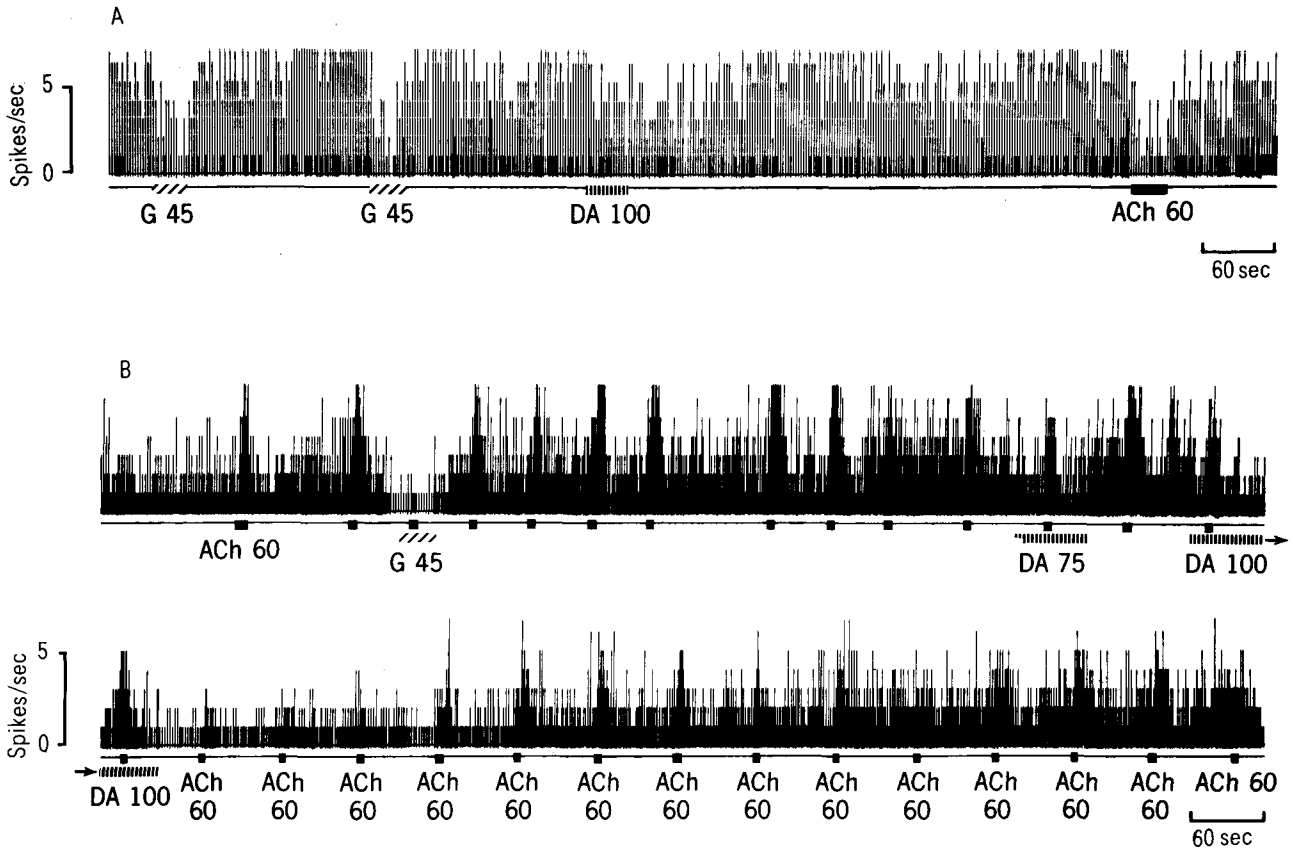
Methods. The experiments were performed on 15 adult cats which were initially anaesthetized with sodium methohexital (10 mg/kg i.v.). 'Encéphale isolé' preparations were prepared by making bilateral electrolytic lesions in A: +2.0 mm; L: 1.5 mm and H: −2.0 to −6.0 mm[17]. Standard microiontophoretic and extracellular recording techniques were employed[16,18]. 5-barrel micropipettes having an overall tip diameter of 4 to 8 µm were filled with the following agents: DA-Cl 0.5–0.8 M, pH 4.0; NE-Cl 0.5 M, pH 4.0; 5-HT oxalate 0.1 M, pH 4.0; ACh-Cl 1.0 M, pH 4.0 and GABA 0.5 M, pH 4.0. The central barrel containing 4 M NaCl (resistance 3–6 MΩ) was used for recording and one of the side barrels (2 M NaCl) as a balancing channel. In this study only visually-driven neurons were analyzed. These cells were found 300–1800 µm below the cortical surface and fired in response to a photic stimulus (Grass PS-20 photostimulator, frequency 0.5/sec or the beam of the oscilloscope).

Results and discussion. The biogenic amines DA, NE and 5-HT were found to inhibit the visually-evoked activity of the majority of cortical neurons (table). This inhibition, obtained with ejection currents of 50–100 nA during 20-

Effects of biogenic amines, GABA and acetylcholine on visually-driven cells*

Biogenic amine						GABA				Acetylcholine			
	Total	↑	↓	=	nt	↑	↓	=	nt	↑	↓	=	nt
Dopamine	35	0	22	13	0	0	34	0	1	19	13	2	0
Norepinephrine	84	1	55	22	6	0	78	0	6	47	17	10	10
Serotonin	42	6	16	14	6	0	42	0	0	11	5	20	6
Total	161	7	93	49	12	0	154	0	7	77	35	32	16

↑, Excited; ↓, Inhibited; =, No effect; nt, Not tested. *These cells were sampled between 300–1800 µm in the occipital (visual) cortex of the cat.



Ratemeter records of the photically-evoked firing rate of 2 cortical neurons. The frequency of firing rates was integrated over 100 msec intervals and the duration of drug application is indicated by the horizontal bars, with the dosage in nA given beneath. *A* A unit sampled at 670 µm was inhibited by GABA (G), dopamine (DA) and acetylcholine (ACh). *B* Shows a neuron recorded during the same penetration, but at 1350 µm deep which showed excitatory responses to repetitive acetylcholine pulses (ACh). This response could be blocked by GABA (G) and dopamine (DA).

30 sec was usually of prolonged duration, lasting 4–6 min and often associated with spike hyperpolarization. In the case of DA the onset of the discharge reduction for each stimulus occurred 30–60 sec from the start of the drug ejection, the inhibition was maximal at approximately 2 min and the cells returned to control responses in about 5 min. The onset of the inhibitory response was more abrupt with NE and 5-HT than with DA (10–30 sec) and reached its maximum within 60 sec but the cells also returned to control levels of firing in about 5 min. Although the inhibitory effects of the amines seemed to be widespread throughout the cortex most of the cells unresponsive to DA, NE and 5-HT were found in the upper cortical layers (300–800 µm) while deeper than 1200 µm, practically all neurons were inhibited. The amino acid neurotransmitter GABA inhibited and hyperpolarized all the neurons tested, regardless of their depth. This effect was obtained with ejection currents of 5–30 nA and was rapid in onset and termination lasting only as long as GABA was ejected[19,20]. At the end of the GABA ejection we often found a 'rebound' increase in firing lasting 5–10 sec. Acetylcholine was tested in 144 visually-driven cells (40–60 nA for 5–15 sec) and of these units 32 (22%) showed no change in their evoked firing and 35 (24%) were inhibited. This inhibitory response of cortical neurons to ACh took the form of a rapid decrease in the number of spikes evoked per stimulus outlasting the duration of ejection by 15–20 sec. The cells inhibited by ACh were usually sampled in the upper cortical layers (300–800 µm)[21,22]. Below 1200 µm the vast majority of cells recorded were excited by the application of ACh[20–22]. This excitation started a few seconds after the beginning of ACh ejection, reached a maximum in about 5–10 sec, returned to control levels in about 1 min and could be blocked or reduced by GABA or by the biogenic amines. The blockade of the ACh-induced excitation by GABA was rapid in onset and ceased as soon as the ejection of GABA was terminated (figure B), the cell immediately recovering its sensitivity to ACh. This was in contrast to the blockade induced by the biogenic amines on the ACh-induced increase in evoked firing, which was of long duration. After treating the neuron with DA, or NE or 5-HT the ACh-induced responses returned to 'normal' only after the 2nd or 3rd ACh ejection (figure B). These results show that the evoked activity of a great number of visual cortical cells may be inhibited by DA, NE and 5-HT and that this inhibitory response differs markedly from that induced upon the same cells by GABA. Furthermore, the long duration of the effects caused by DA, NE and 5-HT on the ACh-induced increase in evoked firing when compared with the GABA blockade of ACh-excitation tends to rule out a simple balance between excitations and inhibitions to explain the suppression of the sensitivity towards ACh by NE, DA or 5-HT. Although the molecular mechanisms underlying biogenic amine and ACh interactions are currently being debated[9,16,20,23–26], the interactions reported here probably call upon mechanisms closely related to the direct postsynaptic actions of these putative neuromodulators.

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Failure of medial forebrain bundle or raphe ablation to alter the daily temperature rhythm of the rat

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Summary. The data presented in the present study suggest that neither the ascending noradrenergic fibres confined to the MFB nor the serotonergic fibres originating in or passing through the mesencephalic raphe are essential for periodicity in body temperature. Both control and experimental groups, i.e., rats subjected to medial forebrain bundle or raphe ablation presented circadian periodicity in body temperature and neither the phase, amplitude or overall mean of experimentals differed significantly from controls.

During the last several years considerable attention has been focused on identifying the central neural sites and/or systems involved in rhythmic neuroendocrine function and associated activity. Frequently implicated areas have been the suprachiasmatic nuclei and the ascending monaminergic systems. Circadian rhythmicity in locomotor activity[1], drinking[1], pineal N-acetyltransferase activity[2] and adrenal corticosterone content[2] have been reported to be abolished after ablation of the rodent suprachiasmatic nuclei. Similarly, the ascending serotonergic fibres have been implicated in a variety of rhythmic neuroendocrine activities. Serotonergic neurons have been reported to participate in the regulation of sleep[3,4] motor activity[5] eating[6] pituitary-adrenal activity[7,8] and LH secretion[9].

Recently we confirmed the earlier report of Moore and Eichler[2] that the 24-h periodicity in pituitary-adrenal function is lost after suprachiasmatic nuclei ablation but we were unable to corroborate a previous report that circadian periodicity in body temperature is dependent upon intact suprachiasmatic nuclei[10]. Rather we observed a 24-h periodicity in body temperature which was not different from that of controls[11].

The above observation, taken with the several reports of monaminergic involvement in biorhythmic phenomena resulted in our focusing on the ascending monaminergic fibres as possible neural systems involved in maintenance of the daily rhythm in body temperature. The present report describes the effect of medial forebrain bundle (MFB) or mesencephalic raphe nuclei (RN) ablation on body temperature. These areas were chosen since they encompass the majority of the ascending noradrenergic fibres or represent the primary origin of the ascending serotonergic system.

Materials and methods. Adult female Sprague-Dawley rats (Charles River, CD) weighing approximately 185 g at surgery were used in this study. Prior to surgery, rats housed 2 per cage, were acclimated for 10 days to conditions of controlled lighting (fluorescent illumination from 04.00 h to 18.00 h) and temperature (26±1 °C). Food and water were available ad libitum.

MFB and RN ablation, carried out under pentobarbital anesthesia, was accomplished using a Kopf Radio Frequency Lesion Generator and accompanying probe positioned according to the stereotoxic coordinates of de Groot[12]. After surgery rats were housed in individual cages. Intact rats served as controls. To familiarize rats to handling and to assess reproductive cyclicity vaginal smears were taken 5 days a week beginning 20 days prior to temperature determination.

80 days after central neural ablation, body temperature was measured every 4 h over a 48-h period using a YSI Tele-Thermometer equipped with a rectal probe. To standardize the protocol the probe was inserted 15–17 mm through the rectum, and recordings were made 20 sec after insertion of the probe.

At autopsy brains of lesioned rats were removed, fixed in formalin and subsequently prepared for histological evaluation of the ablated area. Only rats presenting complete ablation of the MFB and RN are included in this report. Statistics were derived from analysis of variance.

Results. As evidenced in figures 1 and 2, circadian periodicity in body temperature occurred in MFB ($F=5.21$,