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Anatomical correlates of the lateral hypothalamic influence on waking-sleep relationship in the rat

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Abstract. Restricted electrolytic lesions of the lateral hypothalamus (LH) evoke sleeplessness in the rat. The present study was aimed to analyze a possible anatomical substrate of the LH hyposomnia within the hypothalamus. In a group of electrolytically lesioned LH rats the intensity of sleep disturbances, assessed on the basis of EEG records from the neocortex and the hippocampus, was confronted with the localization and the extent of destruction of the LH area and with the topography of known fiber systems of the medial forebrain bundle (MFB). In separate experiments the effects of the destruction of LH cell bodies by means of bilateral ibotenic acid (IBO) injections and inhibition of LH neuronal elements by bilateral muscimol (MUSC) administration were also tested. It was found that pronounced hyposomnia follows electrolytic but not IBO lesions of the LH/MFB area. The effective LH damage might have been localized at every level of its antero-posterior axis, from the preoptic area up to the posterior hypothalamus, suggesting involvement of fiber system(s) rather than a localized group of neuronal pericaria. The most effective lesions transsected projections descending from the preoptic/anterior hypothalamic area, olfactory structures, ventral striatum and the central amygdaloid nucleus as well as fibers connecting LH with the brainstem reticular formation, many of them using GABA as a neurotransmitter. Bilateral MUSC injections caused a dose-dependent, bicuculline-reversible, increase in waking time, most pronounced at a dose of 50 ng, which ressembled the effect of the electrolytic lesion. These results indicate that LH hyposomnia is not attributable to the damage to the intrahypothalamic neurons and suggest the participation of GABA-ergic transmission in LH in waking-sleep regulation.

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Key words: lateral hypothalamus, medial forebrain bundle, lesions, hyposomnia, ibotenic acid, muscimol, EEG

INTRODUCTION

Sleep-waking phenomena are integrated at several levels of the neuroaxis. At the hypothalamic level the preoptic-anterior hypothalamic (POAH) and the posterior hypothalamic (PH) areas were found to be most directly involved. POAH is regarded as a "sleep promoting area" because: (1) it contains neurons selectively active during slow wave sleep (SWS) (Kaitin 1984, Szymusiak and McGinty 1986a, Ogawa and Kawamura 1988, Koyama and Hayaishi 1994) and paradoxical sleep (PS) (Koyama and Hayaishi 1994); (2) damage to POAH causes drastic reduction of the amount of both SWS and PS (Nauta 1946, McGinty and Sterman 1968, Szymusiak and McGinty 1986 b, Sallanon et al. 1989); (3) electrical or chemical stimulation of this area induces SWS (Sterman and Clemente 1962 a,b, Ueno et al. 1982, Garcia-Arraras and Pappenheimer 1983, Mallick and Alam 1992); (4) increased c-fos expression was found in the ventrolateral POAH after long episodes of SWS (Sherin et al. 1996).

On the other hand, PH was postulated to promote cortical desynchronizing activity because this area: (1) contains a population of neurons selectively active during waking and REM sleep (Vanni-Mercier et al. 1984, Sakai et al. 1990, Steininger et al. 1999); (2) has topographically organized projections to all cortical areas (Saper 1985); (3) its destruction or dissection causes lethargy (von Economo 1926) and drastic impairment of cortical activation (Ranson 1939, Nauta 1946, Lindsley et al. 1950).

POAH and PH are functionally connected: (1) local warming of POAH causes decrease in neuronal activity in PH (Krilowicz et al. 1994); (2) insomnia induced by damage to POAH can be reversed by direct injection of muscimol to PH (Sallanon et al. 1989) suggesting that induction of sleep may result from a functional blockade of PH by POAH. Anatomical substrate of this inhibitory influence may constitute the monosynaptic GABA-ergic projections from POAH to PH (Gritti et al. 1994). Extracellular level of GABA in PH is increased during sleep (Nitz and Siegel 1996), and $GABA_A$ agonist injection to PH reverses experimentally induced insomnia (Lin et al. 1989, Sallanon et al. 1989). PH can influence POAH through ascending histaminergic projections; reduction of the amount of SWS was found after local intra-POAH injection of H_2 receptors agonist (Lin et al. 1994).

The lateral hypothalamus (LH) is anatomically situated between POAH and PH and it can constitute a natural "communication channel" between the sleep and waking promoting areas. As was found in a series of our studies on rats (Trojniar et al. 1987, 1990, Jurkowlaniec et al. 1994b, 1996) electrolytic lesions restricted to LH cause hyposomnia not related (Jurkowlaniec et al. 1994a,b) to other behavioral disturbances of the so called „LH syndrome" (Teitelbaum and Epstein 1962) such as aphagia, adipsia and motor restlessness. For the first time the LH hyposomnia effect was described by Danguir and Nicolaidis (1980) in rats partially recovered from massive LH destruction.

LH area is mainly occupied by the medial forebrain bundle (MFB) - the large, longitudinal fiber system connecting the prosencephalic and diencephalic structures with the mesencephalic tegmentum. It contains about 50 pathways, both ascending and descending, which use several neurotransmitters and neuromodulators (Nieuwenhuys et al. 1982) and is relatively poor with neural pericaria (Millhouse 1969). The topographic arrangement of at least some of these pathways and neuronal groups in the rat LH was described by Dutch anatomists (Nieuwenhuys et al. 1982, Veening et al. 1982, Geeraedts et al. 1990 a,b).

The present paper was aimed to analyze a possible anatomical substrate of LH hyposomnia. We took a group of electrolytically lesioned rats from the archives of our laboratory and confronted the intensity of lesion-induced hyposomnia with the localization and the extent of destruction of the LH area. The topography of the most effective lesions was compared with the arrangement of MFB fiber systems as given in the atlas by Nieuwenhuys et al. (1982) and Veening et al. (1982). In the next experiment we examined the possible involvement of LH neuronal pericaria in the LH lesion-induced sleeplessness by the method of bilateral injections of ibotenic acid (IBO) (Winn 1991).

Another approach to the study of the role of the hypothalamic neurons in the regulation of sleep-waking cycle was adopted in the cat by Lin et al. (1989), who induced the hyperpolarization (supposedly mimicking the effect of local chemical lesion) of hypothalamic neurons by means of direct application of muscimol (MUSC) - a $GABA_A$ agonist. The same approach was applied in the present study on the role of LH in the rat.

METHODS

Animals

Sixty male Wistar rats weighing about 250-350 g on the day of surgery were used, 31 of which were subjected

to the bilateral electrolytic lesions of LH, and 11 control animals were sham-lesioned. Ten animals were subjected to bilateral IBO injections, and 8 other rats were bilaterally injected with MUSC.

The animals were kept in individual home cages with laboratory pellets and tap water *ad libitum* in an artificially maintained 12 : 12 hour light/dark cycle. The rats which developed aphagia and adipsia after LH damage were additionally fed and watered by means of a gastric tube, once or twice a day just after completion of EEG recordings.

Surgical procedure

All rats were implanted under Nembutal anesthesia (50 mg/kg) with bilateral recording electrodes in the dorsal hippocampus and over the occipital cortex, earth screw electrode and the reference electrode. Silver wire EMG electrode in the neck muscles were also implanted. Detailed description of the construction and implantation of electrodes was presented elsewhere (Trojniar et al. 1987). Besides, 42 rats were implanted with chronic electrodes for bilateral electrolytic lesions (stainless steel wire, 0.3 mm diameter, insulated on the entire length except for the square-cut tip) and 18 animals with bilateral stainless steel cannulas (15.0 mm long, 0.6 mm in diameter) for intracerebral injections aimed at the region of LH.

Paxinos and Watson (1986) stereotaxic coordinates for LH electrodes were as follows: 1.2-4.5 mm posterior to the bregma, 1.0-2.0 mm lateral to the midline and 7.7-9.0 mm below the surface of the skull. Coordinates for LH cannulas were as follows: 2.8 mm posterior to the bregma, 2.0 mm lateral to the midline and 8.0 mm below the surface of the skull. Recording electrodes in the dorsal hippocampus were implanted 2.4-3.8 mm posterior to the bregma, 2.4-3.0 mm lateral to the midline and 2.5-4.0 mm below the skull surface. Neocortical recording electrodes were screwed 6.0-7.0 mm posterior to the bregma and 2.5-3.5 mm lateral to the midline at a depth of 1 mm below the skull surface.

EEG recording

The animals were allowed about a week recovery period from the surgery, during which they were adapted to the experimental conditions. The rats were put into the recording chamber for the same hours of the day as those of the actual experiment.

After completion of the adaptation procedure, EEG recording began. The recordings were carried out in glass cages measuring 260 x 260 x 400 mm placed in a sound attenuating chamber once a day for 1 h (morning hours) in the electrolytically lesioned and IBO groups, and for 6 h (from 10.00 a.m. to 4.00 p.m.) in the MUSC-injected group. As was proved in our previous study (Jurkowlaniec et al. 1994 b) the one hour morning record is sufficient to diagnose the presence of hyposomnia in LH-lesioned rats. The rats were put into the chamber 1 h before the beginning of the EEG recording to avoid possible arousing effects of moving the animal from the home cage to the recording cage.

The cortical and hippocampal EEG recordings were made using a 16-channel Medicor electroencephalograph (bandpass 0.3-50 Hz). The animals were continuously observed through a camera connected to a monitoring system and their behavior (walking, rearing, probable sleep, etc.) was noted concomitantly with the EEG recording. The baseline EEG was recorded for 3 days before the experimental treatment. In the electrolytically lesioned animals and their sham controls experimental recording started on the day following electrocoagulation of LH and continued for 5-7 days of the first and for 2-3 days of the second postlesion week. In the IBO group EEG was recorded for 3 days following the control injection of the solvent, and for 8 consecutive days and then on day 11 and 14 after IBO injections. In the MUSC-injected group EEG was recorded once after each dose of MUSC.

All EEG records were visually analyzed and counted for the amount of low voltage fast activity in the neocortex and the hippocampus (waking without theta, W), low voltage fast activity in the neocortex accompanied by theta rhythm in the hippocampus (waking with theta, WT), large amplitude irregular activity in the neocortex and hippocampus (SWS) and generalized theta rhythm in the neocortex and hippocampus accompanied by neck muscle atonia (PS). The amount of the particular types of EEG activity was expressed as a percentage of the total recording time or as a percentage change from the baseline. In the IBO and MUSC-injected group the number of episodes of W, WT, SWS and PS, the duration of a single episode and the latency of a first episode of SWS and PS were also calculated in each daily record.

Electrolytic lesions and intracerebral injections

Electrolytic lesions in the experimental animals were performed under light ether anesthesia after completion of the baseline EEG recordings, i.e. about 10 days after

the implantation of electrodes. Lesions were produced by passing 1.0-2.0 mA anodal current for 15-20 s. A sham operated group was treated in the same way except that no current was passed through the LH electrodes.

All intracerebral injections were performed using a Hamilton syringe through the chronically implanted guide cannulas. The needle of the syringe (0.3 mm diameter) extended 0.4 mm past the tip of the guide cannula. Injection time was 1 min, and another minute was allowed before the syringe was disconnected.

Ibotenic acid (RBI) dissolved in phosphate buffer (pH 7.4) was injected bilaterally at a dose of 3 μg/0.5 μl per hemisphere under light (50 mg/kg) ketamine anesthesia (Bioketamine, Biowet). In 6 animals IBO injection was preceded by intra-LH administration of the solvent (0.5 μl per hemisphere). EEG recording started the next day after injections.

Muscimol Hbr (RBI) at a dose of 0 ng (Aqua pro injectione, Polfa), 25 ng, 50 ng and 100 ng /0.5 μl was injected bilaterally according to the same procedure. Each rat received each dose in an ascending order. Seven days interval was allowed between each injection. At the end, 5 animals received bilateral combined injection of 50 ng of bicuculline (Bicuculline methiodide, Sigma) followed 15 min later by 25 ng of muscimol (equimolar doses). Higher

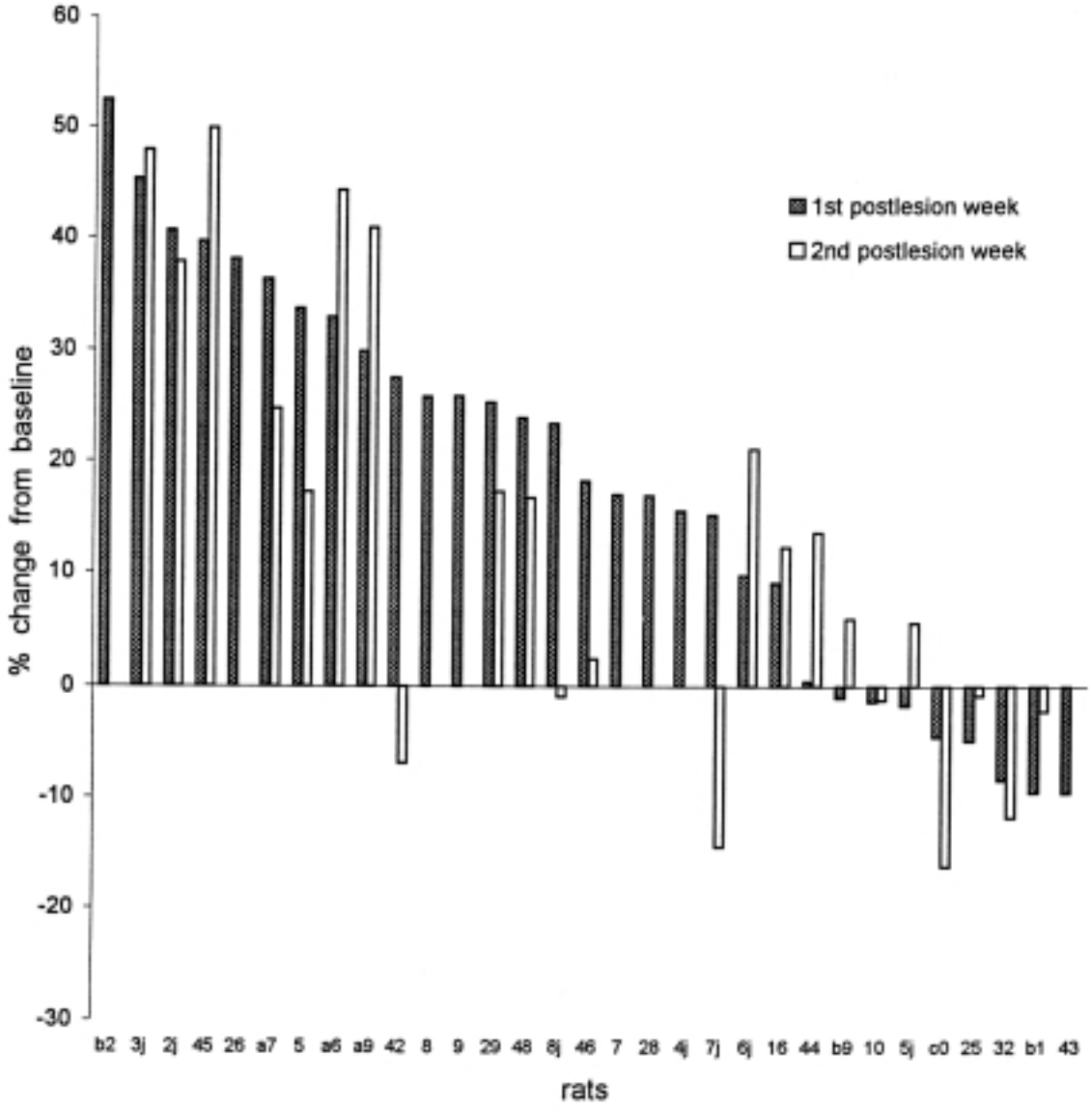

Fig. 1. Waking time expressed as a percentage change from the baseline in all LH-lesioned animals ($n = 31$). Data were averaged across weeks of the experiment and arranged in descending order. Some rats were tested only for one week postlesion.

doses of bicuculline evoked seizures which limited the dose of MUSC subjected to the blockade. The rats were put into the recording chamber immediately after each injection.

Histology

After completion of the experiment the rats were treated with an overdose of anesthetic and then intracardially perfused with 0.9% saline followed by 10% solution of formalin. The brains were removed from the skull and placed in 10% formalin. After fixation brain sections 30-75 μm thick were cut using a frozen tissue technique. The sections were stained with cresyl violet for cell bodies. In IBO rats lesions were identified as areas of neuronal loss and glial proliferation.

The Saper et al. (1979) division of the hypothalamus into the anterior, tuberal and posterior part was used to determine the antero-posterior localization of the lesions and the cannula tips.

Statistics

In the electrolytically lesioned group and the sham control group the postlesion results of the percentage amount of total waking (W+WT), SWS and PS were averaged across weeks of testing and subjected to the two-way ANOVA with factor group and time. Two-way ANOVA with factor distance from the bregma and coronal section of LH was used to assess frequency of damage to the distinguished parts of the LH area. In the IBO group separate one-way ANOVAs were conducted on the percentage amount of W, WT, SWS and PS, on the number and duration of the episodes of the particular patterns of EEG and on latency of SWS and PS with factors treatment (IBO versus solvent) and time (week) after IBO injection. In the MUSC group two-way ANOVA with factors dose and time (hour) after injection was conducted. Findings from ANOVA were further analyzed using Tukey test at $P<0.05$, or Student t-test (paired).

RESULTS

Effect of electrolytic LH lesions on waking-sleep relationship

In the experimental group lesion caused an increase in the amount of waking mainly due to shortening of SWS. Before the lesion, mean percentage distribution of the distinguished patterns of cortical and hippocampal activity in the analyzed EEG samples was as follows: total waking (W+WT), 44.1 ± 1.9%; SWS, 45.5 ± 1.5%; PS, 10.4 ± 0.7%, which did not differ significantly (one-way ANOVA) from the analogous values of the control sham-lesioned group (total waking, 49.8 ± 3.3%; SWS, 42.1 ± 2.6%; PS, 8.1 ± 0.9%).

The postlesion results were averaged across weeks of testing and subjected to the two-way ANOVA with factor group and time postlesion. There was a significant effect of group on the percentage amount of waking ($P<0.0001$) and SWS ($P<0.0001$) but not on PS ($P<0.2$). The waking-sleep distribution in the experimental group during the first and second postlesion week was as follows: total waking, 61.7 ± 2.0 and 56.0 ± 3.2%; SWS, 31.2 ± 1.6 and 36.0 ± 2.7%; PS, 7.1 ± 0.5 and 8.1 ± 0.7%. The effect of week postlesion was significant (total waking, $P<0.0001$; SWS, $P<0.0001$; PS, $P<0.001$). The experimental and control group differed significantly (one-way ANOVA) in the postlesion period in the amount of waking ($P<0.0001$) and SWS ($P<0.0001$) but not PS ($P<0.2$), and the differences concerned the first postlesion week (Tukey test).

The animals of the experimental group differed in the intensity of hyposomnia. Figure 1 shows the individual results of waking time expressed as a percentage change from the prelesion baseline in all LH-lesioned rats. In half of the animals ($n = 15$) the mean increase in waking time exceeded 20% of the baseline reaching even 52.5%, in 5 rats it oscillated between 10-19%, and in 11 others did not exceed ± 10% in the first postlesion week. In the second week an increase of over 20% in waking time was found in 7 animals. In the sham-lesioned group mean change in the waking time was -2.4 ± 4.3% in the first and 2.5 ± 4.3% in the second week, however individual fluctuations on particular days of the experiment might have reached up to -23.3% and 19.0%. On the basis of the intensity of hyposomnia (expressed as a percentage increase in waking time, Fig. 1) rats were divided arbitrarily into 2 groups: those in which lesion-induced increase in waking exceeded 20% of the prelesion baseline, i.e. the value never observed in the control conditions (group I), and those with an increase below 20% (group II).

Anatomical verification of the electrolytic lesions

All the experimented rats had damage to the LH/MFB area. Lesions might have been localized at the level of the preoptic/anterior, tuberal or posterior LH or they

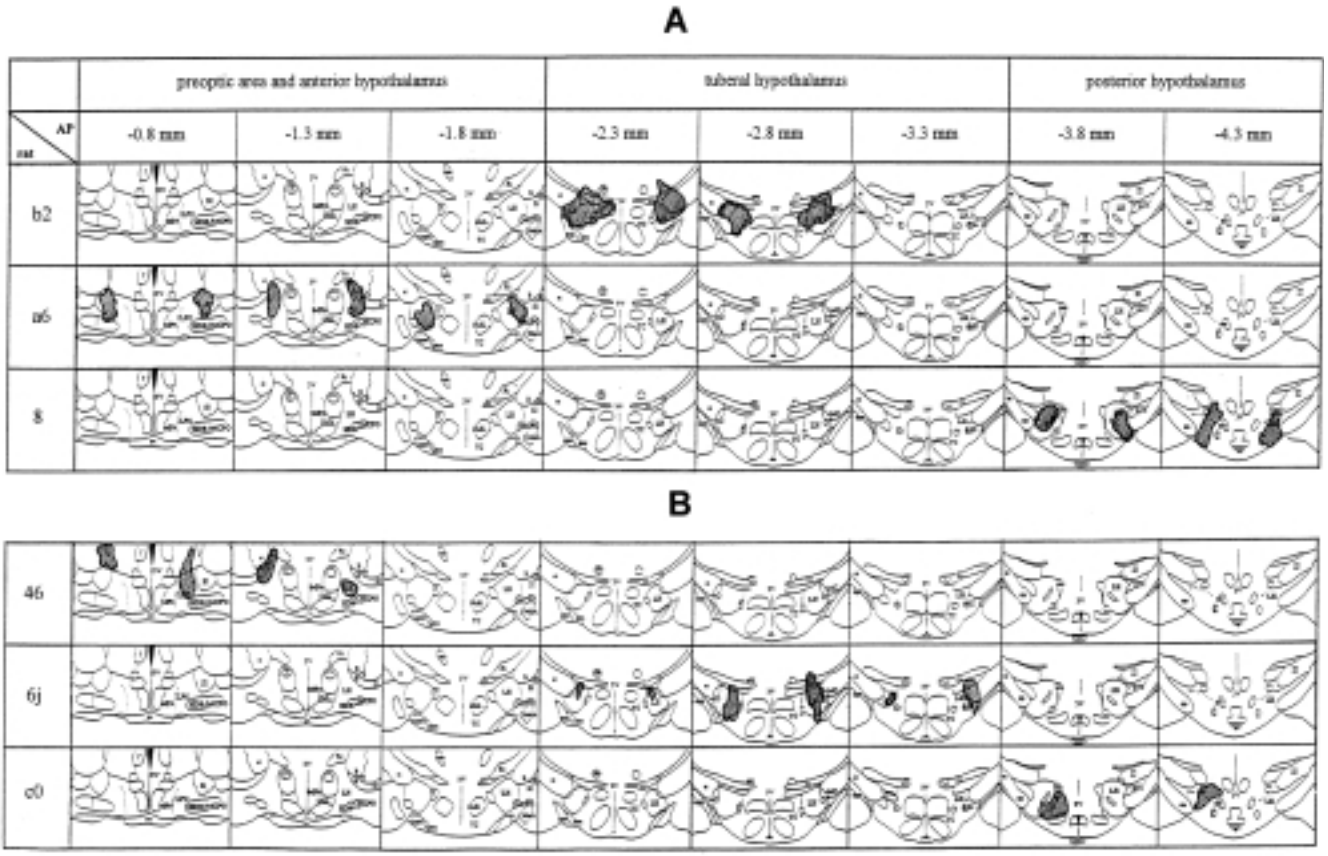

Fig. 2. Anatomical verification of the lesions (shaded areas) in the representative rats of group I showing after the lesion at least 20% (mean) increase in waking time (A) and of the less disturbed group II (B).

rats	preoptic area and anterior hypothalamus						tuberal hypothalamus					posterior hypothalamus				
b2																
3j																
2j																
45																
26																
a7																
5																
a6																
a9																
42																
8																
9																
29																
48																
8j																
AP:	- 0.80	- 0.92	-1.30	-1.40	-1.80	-2.12	-2.30	-2.56	-2.80	-3.14	-3.30	-3.60	-3.80	- 4.16	- 4.30	- 4.52

Fig. 3. Distribution of the lesions (shaded areas) in the antero-posterior plane of LH in group I ($n = 15$) of severely disturbed animals. Rats are presented in order identical as in Fig. 1. AP, distance from the bregma (mm) according to the atlas by Paxinos and Watson (1986).

might have involved two neighboring levels. Occasionally lesions encroached upon the internal capsule, zona incerta, ventral thalamus, fields of Forel or the medial preoptic area. Localization of the lesions in rats representative for the group I and group II is presented in Fig. 2A and B respectively.

The greatest increase in waking (with parallel reduction of sleep) accompanied lesions localized in the anterior and tuberal LH, frequently at the border of these two levels. Effective lesions might also have been localized entirely at the level of the posterior LH. Figure 3 summarizes distribution of the lesions in the antero-posterior plane in the most disturbed rats. As can be seen, pronounced hyposomnia was evoked by lesions localized at each of the distinguished LH levels.

Localization of the lesions in the animals of group II was not critically different and also involved all antero-posterior levels of LH (Fig. 2B). However, in animals with slight or no hyposomnia lesions were usually either very small or unilateral except 3 rats, in which large damage to the anterior and tuberal LH was not basically different from that found in group I.

In order to assess the importance of different fiber systems of MFB, the coronal sections of LH area at each antero-posterior level were divided into 4 squares (Fig. 4 top) and the size and frequency of the damage to the particular squares were evaluated in all animals. In 9 animals lesion destroyed 100% of the coronal section of LH bilaterally, in 7 others 75% of LH was destroyed, about 50% of the area was destroyed in 11 subjects and up to 25% in 4 rats. Some lesions were bilaterally asymmetrical either in the antero-posterior or in the coronal plane. In 9 out of 15 animals of group I, lesions dissected 75-100% of the coronal section of LH.

The frequency of damage to each of the distinguished squares (a, b, c or d) was counted using 16 tables from the Paxinos and Watson atlas (1986) depicting the LH area (distance -0.80 to -4.52 mm from the bregma). Two-way ANOVA (distance from the bregma x square) was conducted on the frequency data in each group separately. In group I, containing the most disturbed animals, significant effect of AP dimension ($P<0.001$) and square ($P<0.022$) as well as significant ($P<0.012$) AP x square interaction was found. Post hoc comparisons (Tukey test) showed the greatest frequency of damage to the anterior LH. Analogous analysis performed in group II revealed significant effect of AP dimension ($P<0.001$) but not the effect of square, with the anterior LH being most frequently damaged.

Student *t*-test (paired) comparison of the frequency of damage to the distinguished squares showed that in group I the most frequently damaged LH areas were (Fig. 4 bottom): the dorso-lateral ($P<0.01$) and ventro--lateral ($P<0.02$) in the anterior LH, dorso-lateral ($P<0.005$) in the tuberal LH, ventro-lateral ($P<0.001$) and ventro-medial ($P<0.01$) in the posterior LH. These areas cover the following sectors of the medial forebrain bundle as described in the atlas by Nieuwenhuys et al. (1982) and Veening et al. (1982): a, a_1, e_1 and d (anterior LH), e, e_1 and e_2 (tuberal LH), a, d and g (posterior LH).

Effect of IBO injections on waking-sleep relationship

The results are presented in Table I. No significant effect of IBO injections and time (week) after injection on the percentage distribution of total waking (W+WT), SWS and PS was found in the analysis of variance (comparison both to the preinjection control and the phos-

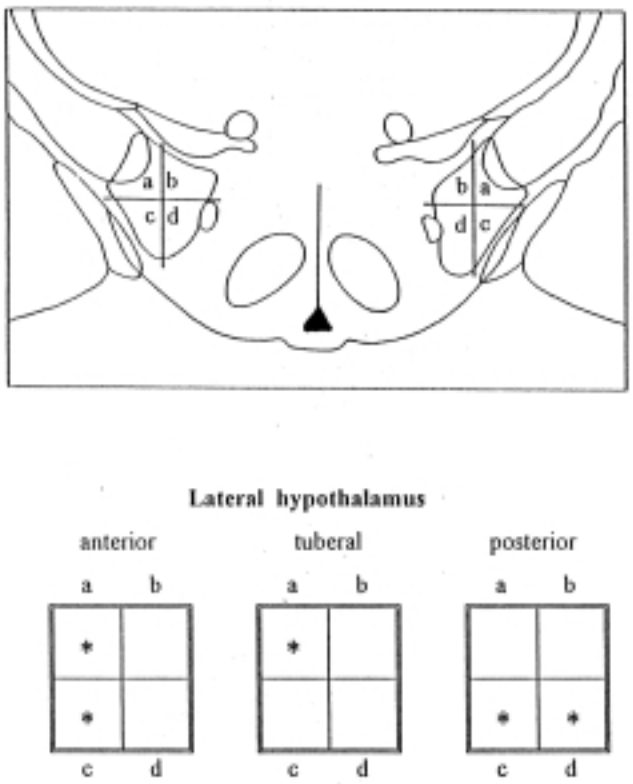

Fig. 4. Coronal sectors of the LH/MFB area which were significantly (asterisks) more frequently damaged in group I of severely disturbed rats. Paxinos and Watson (1986) stereotaxic coordinates of the anterior, tuberal and posterior LH are given in Fig. 3

TABLE I

Characteristics of waking-sleep relations in the IBO-lesioned rats

		Mean ± SE	control (before lesion)	phosphate buffer	1st postlesion week	2nd postlesion week
	W+WT	% of total recording time	48.5 ± 2.7	43.2 ± 3.9	39.8 ± 1.9	36.3 ± 3.3
Waking	W	% of total recording time	44.6 ± 2.5	40.8 ± 4.0	35.8 ± 1.8	31.8 ± 3.0
		number of episodes	33.8 ± 1.6	30.1 ± 1.7	34.3 ±1 .0	32.7 ± 2.3
		duration of a single episode (s)	51.5 ± 3.5	49.4 ± 4.7	38.6 ± 1.6*	36.3 ± 2.6*
	WT	% of total recording time	3.9 ± 0.5	2.5 ± 0.6	4.1 ± 0.3	4.5 ± 0.6*
		number of episodes	10.6 ± 1.1	5.7 ± 1.1[a]	9.3 ± 0.6*	10.7 ± 1.5*
		duration of a single episode (s)	14.2 ± 0.9	14.8 ± 1.9	15.5 ± 0.8	16.2 ± 0.7
Slow wave sleep		% of total recording time	42.9 ± 1.9	46.3 ± 3.2	49.3 ± 1.6	53.5 ± 2.8
		number of episodes	23.3 ± 1.1	26.0 ± 1.9	27.1 ± 0.8	24.3 ± 1.0
		duration of a single episode (s)	71.4 ± 3.8	71.4 ± 7.7	71.4 ± 3.1	84.5 ± 5.7
		latency of the first episode (s)	399.3 ± 68.6	165.6 ± 60.3[a]	159.2 ± 29.7	52.6 ± 14.0
Paradoxical sleep		% of total recording time	8.7 ± 0.9	10.5 ± 1.2	10.9 ± 0.7	10.2 ± 1.1
		number of episodes	4.3 ± 0.4	6.3 ± 0.7[a]	5.8 ± 0.3	5.4 ± 0.6
		duration of a single episode (s)	76.1 ± 6.7	63.1 ± 6.5	72.7 ± 3.7	73.8 ± 6.6
		latency of the first episode (s)	1426.2 ± 171.7	786.9 ± 152.8[a]	968.8 ± 104.6	1114.8 ± 211.4

*, $P<0.05$, significantly different from buffer injection (Tukey test). a, $P<0.05$, difference between the preinjection control and buffer injection (one-way ANOVA).

phate buffer injections). As phosphate buffer affected significantly some parameters (number of episodes of WT and PS and latency of SWS and PS) further comparisons were performed using phosphate buffer data as the baseline. The only significant effect of IBO on the amount of waking and sleep was a slight increase in the percentage of WT in the second postlesion week. An increase in the number of WT episodes and a shortening of W episodes significant for the whole post-IBO period were also noted.

Histological verification of IBO lesions

Figure 5 summarizes the localization and the extent of cytotoxic lesions. The neuronal loss accompanied by a proliferation of glial cells was found in the tuberal and partly anterior LH lateral to the internal capsule invading also the neighboring structures (zona incerta, ventral thalamus). Lesions were usually bilaterally symmetrical.

Effects of MUSC injections on waking-sleep relationship

The results are shown in Table II and in Fig. 6. Bilateral injections of MUSC to LH resulted in a dose-dependent increase in waking time with simultaneous reduction of the amount of SWS and PS. Two-way analysis of variance revealed significant effect of dose and time (hour) after injection on percentage distribution of total waking (W+WT) ($P<0.001$ for both factors), SWS ($P<0.001$ for both factors) and PS ($P<0.001$ for both factors). Dose x time interaction was significant for waking ($P<0.001$) and SWS ($P<0.002$) but not PS. Waking time was increased mainly due to the increase of W ($P<0.001$ for both factors, $P<0.001$ dose x time interaction) but not WT. Tukey test post hoc comparisons showed significant difference between the effect of dose 0 ng and doses 50 and 100 ng of MUSC on percentage amount of total waking, W and SWS, and between 0 ng and 50 ng in the case of PS.

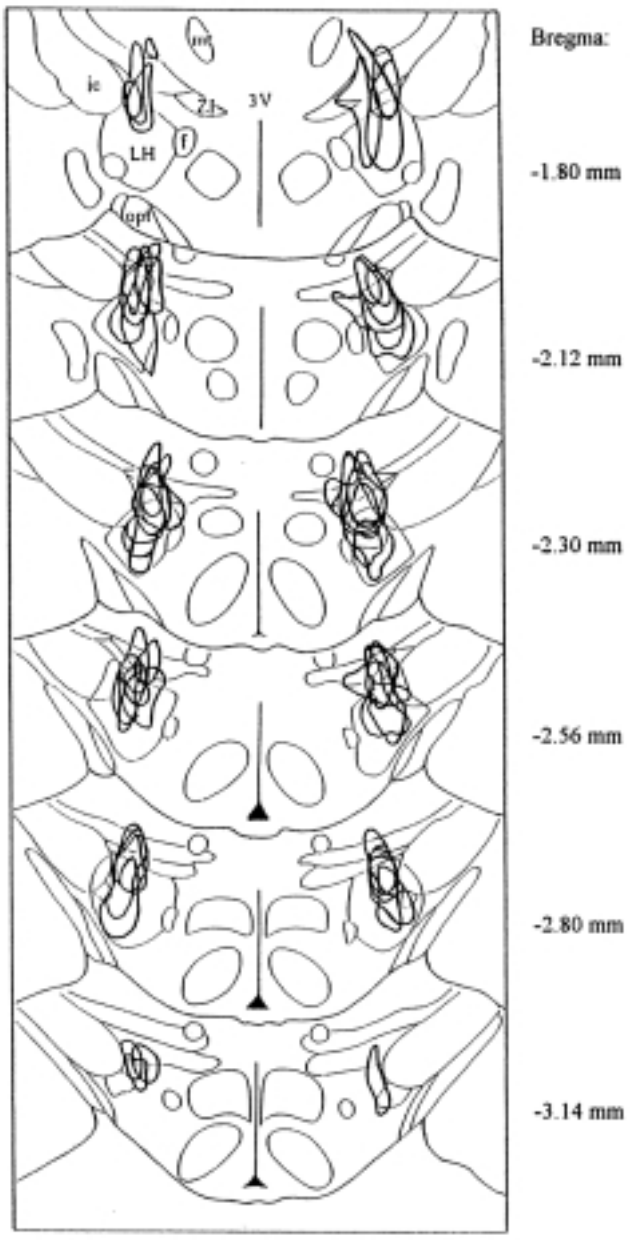

Fig. 5. Extent of cell loss and gliosis within LH in IBO--lesioned rats.

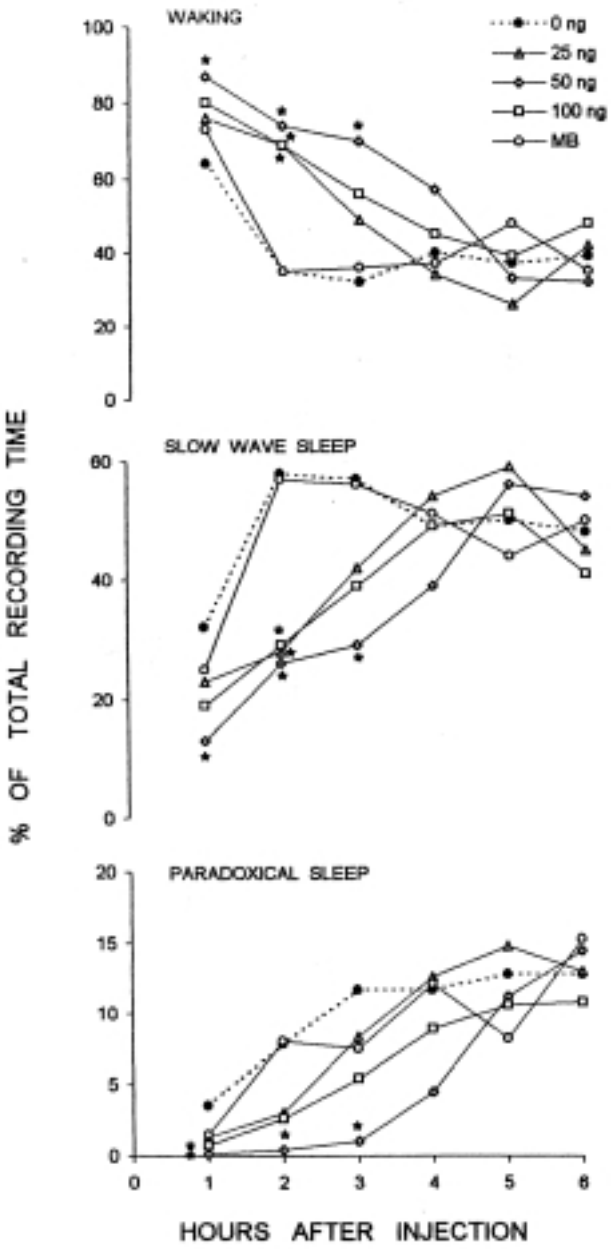

Fig. 6. Time-course of MUSC effect on percentage distribution of total waking (W+WT), SWS and PS. MB, combined injection of 50 ng of bicuculline and 25 ng of MUSC. *, $P<0.05$, significantly different from water (0 ng) injection (Tukey test).

The effect of time after injection in relation to MUSC dose is shown in Fig. 6. High level of waking during the first hour of recording both in the control (0 ng) and in the experimental conditions can be attributed to the arousing influence of the injection procedure. During the next hour this effect subsided to about half of the initial value at 0 ng dose but waking remained significantly (Tukey test, comparison to 0 ng) increased during the next 2 hours at a dose of 50 ng and during 1 h after 25 ng and 100 ng. Reduction of SWS paralleled an increase in waking and was significant for the first 3 hours in the case of dose 50 ng and for the second postinjection hour at doses 25 ng and 100 ng. PS was decreased after MUSC and this effect was significant for the first 3 hours after the injection of 50 ng and for the first hour after 100 ng.

The influence of MUSC on waking-sleep relation was blocked by bicuculline (50 ng of bicuculline against 25

TABLE II

Characteristics of waking-sleep relations in the MUSC-injected rats

		mean ± SE	muscimol 0 ng	25 ng	50 ng	100 ng
Waking	W+WT	% of total recording time	41.0 ± 2.5	49.4 ± 3.3	58.7 ± 3.7*	56.0 ± 3.4*
	W	% of total recording time	38.1 ± 2.3	45.0 ± 3.1	55.3 ± 3.6*	52.3 ± 3.3*
		number of episodes	29.5 ± 1.4	26.6 ± 1.3	25.3 ± 1.6	25.2 ± 1.3
		duration of a single episode (s)	51. 9± 4.4	69.3 ± 7.7[a]	121.7 ± 22.2*	99.0 ± 14.9
	WT	% of total recording time	2.9 ± 0.4	4.4 ± 1.0	3.4 ± 0.6	3.7 ± 0.4
		number of episodes	7.2 ± 0.8	7.6 ± 1.1	7.2 ± 1.3	8.5 ± 0.9
		duration of a single episode (s)	14.2 ± 0.9	15.9 ± 1.1	14.3 ± 1.4	14.6 ± 1.1
Slow wave sleep		% of total recording time	48.9±1.9	41.7 ± 2.6	36.1 ± 3.0*	37.8 ± 2.7*
		number of episodes	23.9 ± 1.2	19.9 ± 1.1	18.4 ± 1.6*	17.5 ± 1.3*
		duration of a single episode (s)	84.3 ± 5.8	75.7 ± 5.0	74.4 ± 6.4	81.1 ± 5.7
		latency of the first episode (s)	1198.0 ± 242.9	1331.6 ± 252.8	1699.6 ± 401.8	2116.4 ± 356.9
Para-doxical sleep		% of total recording time	10.1 ± 1.0	8.8 ± 1.0	5.3 ± 1.0*	6.5 ± 1.1
		number of episodes	5.4 ± 0.5	4.6 ± 0.5	3.0 ± 0.5*	3.1 ± 0.5*
		duration of a single episode (s)	69.6 ± 5.6	62.6 ± 5.9[a]	33.1 ± 5.5*	44.6 ± 5.6*
		latency of the first episode (s)	3542.4 ± 825.5	5138.0 ± 923.5	10430.6 ± 1534.1*	8504.8 ± 2031.2

*, $P<0.05$, significantly different from water (0 ng) injection (Tukey test). a, $P<0.05$, significantly different from dose 50 ng.

ng of MUSC). In no case did the effect of joint injection of $GABA_A$ agonist and antagonist differ significantly from the water injection.

An increase in waking time resulted from marked dose- ($P<0.001$) and time- (0.001) dependent lengthening of W episodes (dose x time interaction $P<0.01$) which appeared significant (Tukey test) for dose 50 ng of MUSC. Two-way analysis of variance showed also a significant effect of MUSC dose on the number of SWS ($P<0.002$) and PS ($P<0.001$) episodes and a duration of PS episodes ($P<0.001$). Effect of time (hour) after injection was significant for a number of episodes of W ($P<0.05$), SWS ($P<0.001$) and PS ($P<0.001$) and for duration of PS episodes ($P<0.001$). No dose x hour interaction was found. Decrease in the number of SWS and PS episodes and shortening of PS episodes were significant for 50 and 100 ng of MUSC as compared to the water injection. Latency to fall asleep was analyzed only in relation to the MUSC dose and it was found significantly longer ($P<0.005$) only for PS (dose 50 ng).

Histological verification of injection cannulas

All cannulas were localized within the area of the medial forebrain bundle in the tuberal LH neighboring the anterior hypothalamus (Fig. 7).

DISCUSSION

The main findings of the present study can be summarized as follows: (1) In the rat pronounced hyposomnia can follow the lesions of the LH/MFB area at every level of its antero-posterior axis, from the preoptic area up to the posterior hypothalamus. Although the effective damage most frequently involved the anterior LH, marked hyposomnia occurred also after lesions situated entirely at the level of the tuberal and posterior LH. (2) Cytotoxic destruction of the neuronal pericaria within LH (mainly tuberal part) did not affect waking-sleep relation and in this respect differed from the effects of the electrolytic lesions of the same area.(3) Bilateral MUSC

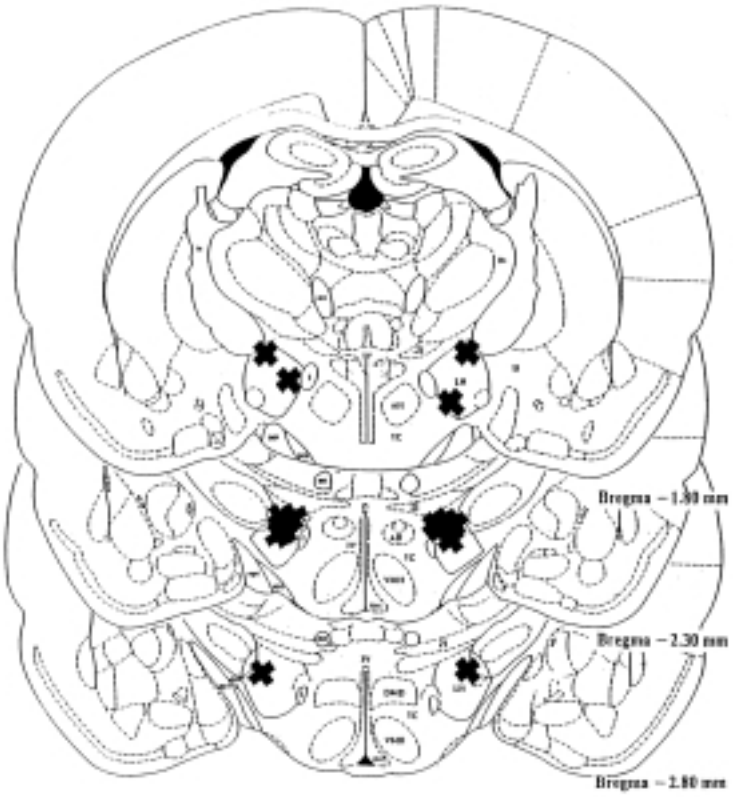

Fig. 7. Histological verification of the cannula tips (crosses) in the MUSC-injected rats.

injections caused a dose-dependent, bicuculline reversible, 3-4 h increase in waking time with simultaneous reduction of SWS and PS. Thus, the effect of MUSC resembled that of electrolytic lesioning of the same LH area (Trojniar et al. 1987, 1990, Jurkowlaniec et al. 1994 b). Increase in waking resulted from lengthening of episodes of quiet waking (without theta rhythm in the hippocampus), and a decrease in SWS resulted from a reduction in the number of SWS episodes. The fall in the percentage amount of PS was caused by a reduction of both the number and duration of its episodes. Latency of PS was also increased.

The fact that effective hyposomnia-inducing electrolytic LH lesions might have been situated at all antero-posterior levels of the LH/MFB area suggests that an unidentified fiber system(s) of the bundle rather than (a) localized group(s) of neuronal pericaria is (are) relevant to the observed sleep disturbances. This assumption is supported by the lack of the effect of cytotoxic destruction of LH neuronal pericaria by means of IBO on sleep-waking distribution both in the rat (present study) and in the cat (Denoyer et al. 1991). Bilateral injection of 2 μg of IBO approximately at the same LH level in the hands of Markowska et al. (1985) was sufficient to evoke some symptoms of the "LH syndrome" (mild ingestive and sensorimotor impairments) and higher doses appeared lethal for the rat. In the present experiment hypophagia of moderate intensity was observed for a few days following IBO. In the EEG experiments, Denoyer et al. (1991) did not find any marked influence of IBO injection to the tuberal and posterior LH of the cat on the amount of waking except short-lasting hypersomnia immediately following the injection.

The question is which one(s) out of over 50 fibers systems of the MFB may be considered as a possible substrate of lesion-induced hyposomnia. In the present study the most effective lesions transsected the bundle in the sectors labeled by Nieuwenhuys et al. (1982) and Veening (1982) in their atlas of the rat's MFB as a, a_1, e, e_1, e_2, d and g. Topographical analysis of the overlap of the hyposomnia-inducing lesions with the course of different fiber components within LH point to the descending projections from the preoptic/anterior hypothalamic area, central amygdaloid nucleus and olfactory structures as the most consistently destroyed. However, due to the complexity of MFB the lesions damaged many more of its fiber constituents including descending striatal (both ventral and dorsal) and cortical, ascending ventral tegmental, parabrachial (Veening et al. 1982), serotonergic from the raphe nuclei (Ungerstedt 1971),

and also others that are difficult to trace at the present stage of the analysis.

Certain amygdaloid nuclei have been reported to synchronize cortical EEG (Kreindler and Steriade 1964) and damage to the amygdaloid projections within MFB can potentially contribute to the LH-hyposomnia. The same concerns the ascending serotonergic fibers (Jouvet 1972), although no direct correlation between destruction of the raphe system and sleep-waking cycle was found (Vanderwolf 1988). However, the more probable impact may have dissection of fibers descending from the POAH and the basal prosencephalon including the olfactory tubercles and the ventral striatum. All these structures contain a relatively large number of GABA--ergic neurons which can be retrogradely labeled from the posterior hypothalamus (Gritti et al. 1994). They may function to dampen activity of waking-promoting area of the caudal diencephalon (Lin et al. 1989, Sallanon et al. 1989). Still another fiber system of possible relevance for waking-sleep regulation which undoubtedly was dissected by all effective lesions is a descending projection from LH to the tegmental pedunculopontine nucleus and the parabrachial nucleus (Luiten et al. 1987), which constitute a mesencephalic--pontine component of the ascending reticular activating system (see Steriade and McCarley 1990). Analogous brainstem projections may pass through LH from the POAH since many sleep-active neurons of this area can be antidromically activated from the midbrain reticular formation (Szymusiak and McGinty 1989a) and electrical stimulation of POAH inhibits the activity of sleep-related neurons in the midbrain (Lineberry and Siegel 1971, Bremer 1973, Szymusiak and McGinty 1989b). Our lesions must have destroyed also ascending projections (some of them histaminergic) from PH to POAH (Garbarg et al. 1976, Watanabe et al. 1984, Lin et al. 1994). However, the fact that our LH lesions did not evoke significant hypersomnia may suggest, at least in the rat, a relatively greater impact in the regulation of waking-sleep relationships of waking-inhibiting elements possibly those arising from the POAH and descending to the lower brainstem waking-promoting centers than those going in the opposite direction.

In the present study (similarly to that of Lin et al. 1989) we used local application of MUSC treated as a suppressor of neuronal activity supposed to mimic localized chemical lesions. However our findings do not fit in with the results of Lin et al. (1989) on cats, who injected MUSC at different points of the LH antero-posterior axis and found the hypersomnia effect at the level of the tuberal and posterior LH. Pronounced increase in wakefulness followed only injections localized in the preoptic/anterior hypothalamic area and the most posterior LH neighboring the midbrain tegmentum. In the present experiment the injections were usually centered in the tuberal LH and we have never observed hypersomnia, an increase in wakefulness occurred instead. The diffusion of MUSC to the preoptic/anterior hypothalamic area, i.e. the region where MUSC suppressed sleep in the studies of Lin et al. (1989) can be excluded as a possible source of this discrepancy because similar diffusion must have occurred also to the most posterior loci from which the hypersomnia effect was observed. At present it would be safe to attribute the inconsistency of MUSC effect in cats and rats to possible species-specific differences in the LH circuitry engaged in waking-sleep regulation. Such differences were found in the cholinergic control of REM sleep (Gnadt and Pegram 1986) and in the sensitivity of waking-promoting histaminergic system (Kiyono et al. 1985, Monti et al. 1986, Lin et al. 1988).

Another problem is the inconsistency of the effect of IBO and MUSC. The lack of the effect of IBO suggests that the impact of LH neurons in waking-sleep regulation is meaningless, which supports the conclusion deriving from the electrolytic lesion experiment. On the other hand, suppression of LH neuronal activity by enhancing GABA-ergic transmission evoked pronounced hyposomnia. It is worth noticing that in the studies on cats performed in Jouvet's laboratory (Lin et al. 1989, Sallanon et al. 1989, Denoyer et al. 1991) no marked influence of intrahypothalamic IBO injections on sleep--waking cycle was found, whereas at the very same loci MUSC evoked a pronounced effect. It can be hypothesized that in LH, MUSC affects not only internal GABA-ergic or GABA-ceptive neurons but also acts on GABA-ergic synapses deriving from extrahypothalamic sources. A GABA-ergic inhibition of LH elements may originate for example in the nonspecific thalamic nuclei (Barone et al. 1994). Such inhibitory synapses may be localized on GABA-ergic axons connecting the preoptic/anterior with the posterior hypothalamus (Gritti et al. 1994) and enhancement of their activity by MUSC can lead to disinhibition of the posterior hypothalamic "waking center" and, as a consequence, to hyposomnia. Experimental verification of this hypothesis as well as possible interspecies differences between the rat and the cat waking-sleep regulation require further study.

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