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MORPHOLOGICAL SUBSTRATUM OF LATERAL HYPOTHALAMIC APHAGIA AND ADIPSIA: A MAPPING STUDY IN THE RAT

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Abstract. The purpose of the experiment was to map an area involved in the regulation of food and water intake within the region of the lateral hypothalamus (LH), the adjacent subthalamus and antero-ventral midbrain. The strategy was to make a series of electrolytic lesions, varying in size and localization and to correlate the size of the damage to particular structures occupying the region with the presence and intensity of ingestive disturbances. The highest correlation was found between the presence and duration of aphagia and adipsia and the size of damage to the tuberal part of LH. Even large lesions localized anteriorly or posteriorly to this region gave only moderate ingestive deficits. Surprisingly, total or almost total disruption of the nigro-striatal bundle at the level of the anterior or posterior LH evoked only slight, if any, disturbances in food and water ingestion. No correlation was found between the duration of aphagia and adipsia and the size of damage to zona incerta, fields of Forel, capsula interna, lemniscus medialis, ventral tegmental area and substantia nigra. Our results did not confirm pharmacological data concerning dopamine depletion-induced aphagia and adipsia. It seems that the role of the nigro-striatal system for LH ingestive impairments is still to be cleared up.

INTRODUCTION

Bilateral destruction of the lateral hypothalamus (LH) produces severe syndrome in which the cardial symptoms consist of impairments in food and water ingestion, taking the form of complete aphagia and adipsia when lesions are large enough (2, 40). Anatomically, the lateral hypothalamus is an extremely complex structure. It contains at least 50 ascending and descending fiber systems, as well as a number of neurons of diverse connectivity (17, 24, 44). After 39 years of investigation it is still uncertain which morphological components of LH are responsible for ingestive disturbances following its destruction. The very first concept of feeding and drinking centers located within LH (2, 20, 41) was in the 1970 s substituted by the idea that many symptoms of LH syndrome, including aphagia and adipsia, result from a disruption of the nigro-striatal dopaminergic fibers ascending from the midbrain to the prosencephalon via the medial forebrain bundle (43). A bulk of experimental data was gathered in support of this view (4, 6, 14 - 16, 25, 26, 35, 37, 48, 49).

The involvement of neurons intrinsic to LH is, however, still under consideration. Electrophysiological studies showed that LH neurons respond to the sensory (32) and metabolic (27) features of food as well as to stimuli associated with cellular (28) and extracellular (11, 38, 39) dehydration. Selective destruction of LH neuronal somata by means of kainic (9, 36, 46) and ibotenic (47) acids produces ingestive abnormalities resembling the effects of electrolytic lesions. On the other hand, there are studies which question the importance of the nigral dopaminergic neurons for eating and drinking (10, 12). Only a slight reduction of food and water intake during the immediate postoperative period, and no change in body weight, were found after virtually complete radio-frequency lesions of the pars compacta of the substantia nigra. Therefore the problem of localization of ingestive functions within the LH is still open to investigation.

The purpose of the present experiment was to map an area involved in the regulation of food and water intake within the region of the lateral hypothalamus, subthalamus and adjacent antero-ventral midbrain. The strategy was to make a series of electrolytic lesions varying in size and localization and to correlate the size of the damage to particular structures occupying the region with the presence and intensity of ingestive disturbances resulting from the lesion.

MATERIAL AND METHODS

The experiment was carried out on 153 male Long Evans rats weighing 230-460 g on the day of surgery. The animals were housed in indi-

vidual home cages in an artificially maintained 12 : 12 hours illumination cycle. 139 rats were subjected to bilateral, electrolytic brain lesions (experimental group). Sham operation was performed in 14 animals (control group). Brain lesions were made according to the standard stereotaxic technique using stainless steel electrodes of 0.3 mm in diameter insulated on the entire length with the exception of the flat cut tip. During sham operation the skull bones were exposed, holes were drilled in the skull, but no electrode was lowered into the brain. All surgeries were performed under Nembural anesthesia (20 mg/kg).

Because the strategy of the experiment was to make the series of overlapping lesions along the whole body of the lateral hypothalamus and the adjacent structures, stereotaxic coordinates of the electrodes differed in particular animals and ranged from 1.3 to 3.0 mm posterior to the bregma; 1.3 - 1.8 mm lateral to the midline; 8.4 - 9.1 mm below the skull surface (with lambda 1 mm below bregma). The antero-posterior (AP) distribution of the electrodes' placements was as follows: anterior LH (AP 1.3), $n = 6$; tuberal LH (AP 1.4 - 2.0), $n = 41$; posterior LH and/or anterior midbrain (AP 2.1 - 3.0), $n = 68$. In 24 rats electrodes were aimed at the subthalamic region (above the tuberal and posterior LH). Lesions were performed by passing 1.0 - 2.0 mA anodal current for 10 - 15 s.

For 3 - 10 days before the surgery and during the entire postlesion period food and water intake and body weight were measured for each animal daily. Always at the same time (10 : 00 a.m.) a weighed portion (70 g) of rat chow pellets was placed in a metal feeder in the animal cage. Next day, the pieces of food that fell onto the tray beneth the cage, and uneaten pellets within the cage, were weighed (care was taken they were properly dryed) and substracted from the total weight of pellets given the day before, in order to determine 24 h food intake. Water (60 ml) was available in calibrated glass-drinking tubes.

The rats which were aphagic and adipsic or extremely hypophagic and hypodipsic for more than 2 days were artificially fed and watered once or twice daily by means of a gastric tube.

Ingestive disturbances in particular subjects were quantitatively characterized by a number of days of aphagia and adipsia and/or hypophagia and hypodipsia, maximal loss of body weight (expressed in a percent of preoperative weight) and by duration of body weight loss (in days). Most of the rats were tested daily until the recovery of feeding and drinking to preoperative values. Animals which developed the most severe ingestive disturbances (aphagia, and/or adipsia for longer than 8 days) were sacrified shortly after they started to gain weight because they were not expected to recover completely within a reasonable time in-

tended for this kind of experiment (according to Teitelbaum and Epstein (40) feeding disturbances may last even for 80 days after LH damage and drinking may not recover completely up to 500th postlesion day).

After completion of the experiment the rats were treated with an overdose of ether, brains were removed from the skull and stored in 10% formalin. They were transferred to 30% sucrose solution 48 h before histological preparation. Brain sections 30 μm thick were cut using a frozen tissue technique. The sections were stained with cresyl violet for cell bodies according to Nissl method. The exact location and the extent of the damage were determined under a light microscope. The lesions were superimposed on plates taken from the stereotaxic atlas by König and Klippel (13). Localization of the lesions within the nigro-striatal dopaminergic system and dorsal noradrenergic bundle were assessed on the basis of atlases by Ungerstedt (42) and Palkowitz and Jacobowitz (29, 30). The topography of mezo-limbic pathways was taken from data given by Simon et al. (34).

The extent of the damage to the particular structure was considered to be small (+) when at least 75% of the volume of the structure was spared by the lesion; medium-sized when 25 - 50% (++) of the structure was destroyed, and large when 50 - 75 (+++) or 75 - 100% (++++) of the total structure was destroyed.

Numbers indicating percentage of the volume of the given structure destroyed by the lesion were taken for analysis of correlation between the size of damage and the duration of aphagia and adipsia.

RESULTS

Control group

In the sham operated group ($n = 14$) the unspecific effect of surgery on food and water intake and body weight was evaluated. Decrease in food intake, in comparison to the preoperative baseline, was observed during 1 - 5 days (most frequently during 2 days) immediately following the surgery, one day aphagia was found in 2 rats. Acute reduction of food consumption (to less than 10 g daily) never exceeded the first postoperative day. Water intake was diminished for 0 - 3 days (usually 1 day); total adipsia never occurred. Maximal loss of body weight in particular subjects ranged from 0 to 8% (usually 1%). Body weight gradually decreased during 0 - 7 days (most frequently 1 - 3 days).

On the basis of the data gathered in the control rats, every decline in food and water intake exceeding 5 and 4 days respectively found in the experimental group, was regarded as a specific effect of the brain lesion.

Experimental group

Daily food intake and body weight. Out of 139 rats subjected to brain lesions, impairments in food intake of different intensity were found in 81 animals. In the remaining 58 rats, a decrease in daily food consumption did not exceed the changes found in the control group. Out of 81 disturbed rats, 65 were used for the mapping study. The rest was discar-

TABLE I

Frequency of damage to the particular diencephalic and mesencephalic structures in rats showing impairments in daily food intake (+) and in animals undisturbed (—) after LH lesions

Brain region	Impairments in daily food intake	Number (and %) of animals bearing damage to the given area			Number (and %) of animals without damage to the given area
		total	including lesions		
			bilateral	unilateral	
Lateral hypothalamus (anterior)	+	14 (21.5)	12 (18.4)	2 (3.1)	51 (78.5)
	—	2 (4.2)	2 (4.2)	0	46 (95.8)
Lateral hypothalamus (tuberal)	+	42 (64.6)	31 (47.7)	11 (16.9)	23 (35.4)
	—	13 (27.1)	10 (20.8)	3 (6.3)	35 (72.9)
Lateral hypothalamus (posterior)	+	45 (69.2)	34 (52.3)	11 (16.9)	20 (30.8)
	—	23 (47.9)	21 (43.8)	2 (4.2)	25 (52.1)
Zona incerta	+	54 (83.1)	36 (55.4)	18 (27.7)	11 (16.9)
	—	34 (70.8)	17 (35.4)	17 (35.4)	14 (29.2)
Fields of Forel	+	40 (61.5)	26 (40.0)	14 (21.5)	25 (38.5)
	—	17 (35.4)	12 (25.0)	5 (10.4)	31 (64.6)
Subthalamic nucleus	+	31 (47.7)	14 (21.5)	17 (26.2)	34 (52.3)
	—	11 (22.9)	5 (10.4)	6 (12.5)	37 (77.1)
Capsula interna and/or cerebral peduncle	+	34 (52.3)	17 (26.2)	17 (26.2)	31 (47.7)
	—	20 (41.7)	8 (16.7)	12 (25.0)	28 (58.3)
Lemniscus medialis	+	26 (40.0)	17 (26.2)	9 (13.9)	39 (60.0)
	—	19 (39.6)	14 (29.2)	5 (10.4)	29 (60.4)
Substantia nigra	+	14 (21.5)	9 (13.9)	5 (7.6)	51 (78.5)
	—	14 (29.2)	6 (12.5)	8 (16.7)	34 (70.8)
Nigro-striatal system: pars compacta of the substantia nigra and N-S bundle	+	61 (93.8)	51 (78.5)	10 (15.3)	4 (6.2)
	—	36 (75.0)	28 (58.3)	8 (16.7)	12 (25.0)
Ventral tegmental area	+	15 (23.0)	10 (15.3)	5 (7.6)	50 (76.9)
	—	11 (22.9)	9 (18.7)	2 (4.2)	37 (77.1)
Mezo-limbic system: VTA, ventral and medial mezo-limbic pathways	+	63 (96.9)	58 (89.3)	5 (7.6)	2 (3.1)
	—	38 (79.2)	32 (66.7)	6 (12.5)	10 (20.8)

ded, either because of developing dysfunctions of the gastro-intestinal tract, which by themselves influenced the food intake on or because of a difficulty in getting the good quality histological verification (e.g. in cases when rats died during the night, which made a quick fixation of the brain impossible).

Depending on the parameters of the lesions various degrees of disturbances in food intake were found in different animals. 13 rats were only hypophagic (6 - 18 days postoperatively), in 10 — one day aphagia was followed by a period of hypophagia. 2 - 12 day total aphagia was found in 42 rats, which was followed by a period of decreased food intake, the duration of which had not been determined for the most distur-

TABLE II

Anatomical verification of the lesions and feeding and drinking deficits in the example rats ($n = 23$) chosen from

Rat No.	Lateral hypothalamus						Subthalamus					
	Anterior (A 6360-5150)		Tuberal (A 4890-4110)		Posterior (A 3990-2790)		Zona incerta		Fields of Forel		Subthalamic nucleus	
	R	L	R	L	R	L	R	L	R	L	R	L
EEG 44	+++	++		+			+					
EEG 48	+++	+++										
H 64			+	+			+	+				
H 17			+	+	+	++	+	+		+		
IP 15			+	++	+	+		+				
H 11			+	++			+	+	++	+		
H 12					++	++	++	++	++	+++	+	
Ko 532					++++				+	+	+++	
H 22												
Ko 539	+	++	++++	+	++++						+	
IP 11			++	++			+++	++				
IP 107					++	+++	++	++	+	++	+	
EEG 9			++	++	+++	+++	+		+	++		
Ko 531							+++	+++	+++	+++	++	++
Ko 516			++	+++	++++	++	+	+	++	++	+++	+++
Ko 518			++	++	++	+++	++	+	+	+	++	+
Ko 549			+++	+++			++	++				
IP 105						+						
EEG 30	++	+	+++	+++				+				
Ko 521			+++	+++	++++	+++	+	+	++	++	++	+
Ko 512	+++	+++	++++	+++	++++	++++	+++	+++	+++	+++	+++	+++
Ko 515	+	+++	+++	++++	++	+++	+	+	+	+		++
Ko 513	+++	+++	++++	+++	++++	++++	+++	+++	+++	+++	+++	+++

bed animals because of its expected permanence. In those animals in which the number of days of aphagia as well as hypophagia was counted, unexpectedly no correlation was found between the duration of both stages of LH syndrome ($r = 0.15$).

Decrease in body weight was compatible with the intensity of ingestive impairments, and ranged, in particular subjects, from 8 to 30% of the preoperative value. Duration of body weight loss ranged from 3 - 12 days (under tube feeding in the most disturbed animals).

Analysis of lesions in disturbed animals and correlation between aphagia and the brain damage. Lesions were localized along the entire length of the ventro-lateral diencephalon from the preoptic area to the mammil-

the group showing ingestive disturbances after the brain damage (R, right hemispheriae; L, left hemispheriae)

Midbrain				Lemniscus medialis		Cerebral peduncle (medial part)		Ascending pathways				Days of aphagia (hypophagia)	Days of adipsia (hypodipsia)
Substantia nigra		Ventral tegmental area						Nigro-striatal bundle					
								diencephalic part		midbrain part		Mezo-limbic pathways (ventral and medial)	
R	L	R	L	R	L	R	L	R	L	R	L	R	L		
							+	++++	+++			++	+++	0 (8)	0 (4)
								+++				+++	++++	1 (6)	0 (1)
												+	+	0 (7)	0 (19)
									+			+	++	1 (18)	1 (22)
						+++	+	+	++				+	0 (7)	3 (7)
								+	++			+	+	0 (8)	1 (14)
		+	+	+	+			+	++			++	+	1 (8)	1 (14)
++						++		++	+++	++	++	+	+++	1 (9)	0 (9)
+		++	++	++	+++					++	++	++	++	0 (6)	0 (5)
							+++		++			+++	+++	2 (5)	0 (5)
								++				+	+	2 (4)	2 (10)
+	+	++	++			+		+++	++	+	+	++	++	2 (5)	3 (5)
								+++	+++			+++	+++	3 (10)	3 (10)
+	+			++	++			++	++++			++	++	3	5
		+	+	+	+	++	++	++++	++++			++	++	4	4
					+	+	++	++++	++++			++	++	5	6
						+	+	++++				+		6	12
++	++		+	+	+++	++		++	++	++	++	+	+	6 (8)	5 (6)
						+		+++	+++			++	+++	7 (12)	9
								++++	++++			++	++	10	4
							++	++++	++++			++++	+++	11	14
							+	++	++++			+++	+++	12	14
								++++	++++			++++	++++	12	4

TABLE III

Anatomical verification of the lesions in the example rats ($n = 17$)

Rat No.	Lateral hypothalamus						Subthalamus					
	Anterior (A 6360-5150)		Tuberal (A 4890-4110)		Posterior (A 3990-2790)		Zona incerta		Fields of Forel		Subthalamic nucleus	
	R	L	R	L	R	L	R	L	R	L	R	L
EEG 45	+++	+++					+					
EEG 29	++	+	+++				++					
EEG 16			++	+			+					
EEG 27			+	+				+				
Ko 526			+	++	++	++					+	+
Ko 523			++	+	++++	++			++		++	+
H 27					+	+	+	++				
Ko 525					++	++			+	++	++	
IP 17					++	++	++	+++	+	+		
EEG 8					+++	+++			+	+		
H 46					+++	+++	+	+	+	+		
Ko 519					+	+	+++	+++	+++	+++	++++	++
IP 101					+++	++	+++	++	+	+	+	+
IP 7							++	++				
IP 102												
IP 106							+++	+++				
EEG 22							++	+++				

lary region, and in some animals, in the antero-ventral part of the midbrain tegmentum. Out of 65 rats of this group, in 58 (82.2%) lesions involved the LH either unilaterally (7 rats; 10.8%) or bilaterally (51 rats; 78.4%). Seven animals had lesions entirely outside LH in the zona incerta (2), or in the midbrain (5). Table I summarizes the frequency of damage to the particular di- and mesencephalic structures. Besides LH, the most frequently lesioned structures were: the zona incerta (83.1% of rats), nigro-striatal (93.8%) and mezo-limbic (96.9%) systems.

In particular subjects lesions of LH could have involved its anterior, tuberal or posterior part, two of these regions or the entire length of LH. At the given level of the antero-posterior axis, lesions could have damaged only a small part up to the total of the medio-lateral dimention of the area. The same concerns other structures under consideration.

Table II specifies the presence and size of lesions of particular brain structures in 23 disturbed animals, representing the main types of brain damage together with resulting feeding and drinking impairments. For-

undisturbed after brain damage (*R*, right hemispheriae; *L*, left hemispheriae)

Midbrain				Lemniscus medialis		Cerebral peduncle (medial part)		Ascending pathways					
								Nigro-striatal bundle				Mezo-limbic pathways (ventral and medial)	
Substantia nigra		Ventral tegmental area						diencephalic part		midbrain part			
R	L	R	L	R	L	R	L	R	L	R	L	R	L
						+	+	+++	+			+++	+++
						+++		++++					+++
												+	+
								+	++			+	+
							+	+	+++			+	+
						++	++++	+++	++			++	++
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								+++	++	+	+	++	++
						+++	+	++	++			++	++
+	++	++	+	++		++		+++	++	+	++	++	++
	+			++	++		++	+	++		+		
++				++	+	++				+++		+	
++	++	++	+		+++					++	++	++	++

mations which were damaged only occasionally (e.g. thalamic nuclei, dorsal noradrenergic bundle, red nucleus, praetectal area) were omitted in the table. Lesions of the fornix column are not shown because their irrelevance for ingestive disturbances has already been proved earlier (23).

Generally, in a group of animals showing one-day aphagia or only hypophagia, small lesions within the postero-lateral LH which encroached upon the adjacent subthalamic structures prevailed. Tuberal part of LH was damaged in 9 rats, but the lesions were either unilateral or bilaterally small (not exceeding 50% of the frontal section of the area). In 2 animals extensive damage involved the anterior part of LH. Extrahypothalamically, lesions partially destroyed the posterior subthalamus and/or the anterior midbrain. Damage to the nigro-striatal system differed greatly in particular rats, ranging from total sparing to almost total disruption of the nigro-striatal bundle at the level of the anterior LH. The mezo-limbic pathways were disrupted in the vast majority of rats, although the degree of damage differed in particular subjects. In general,

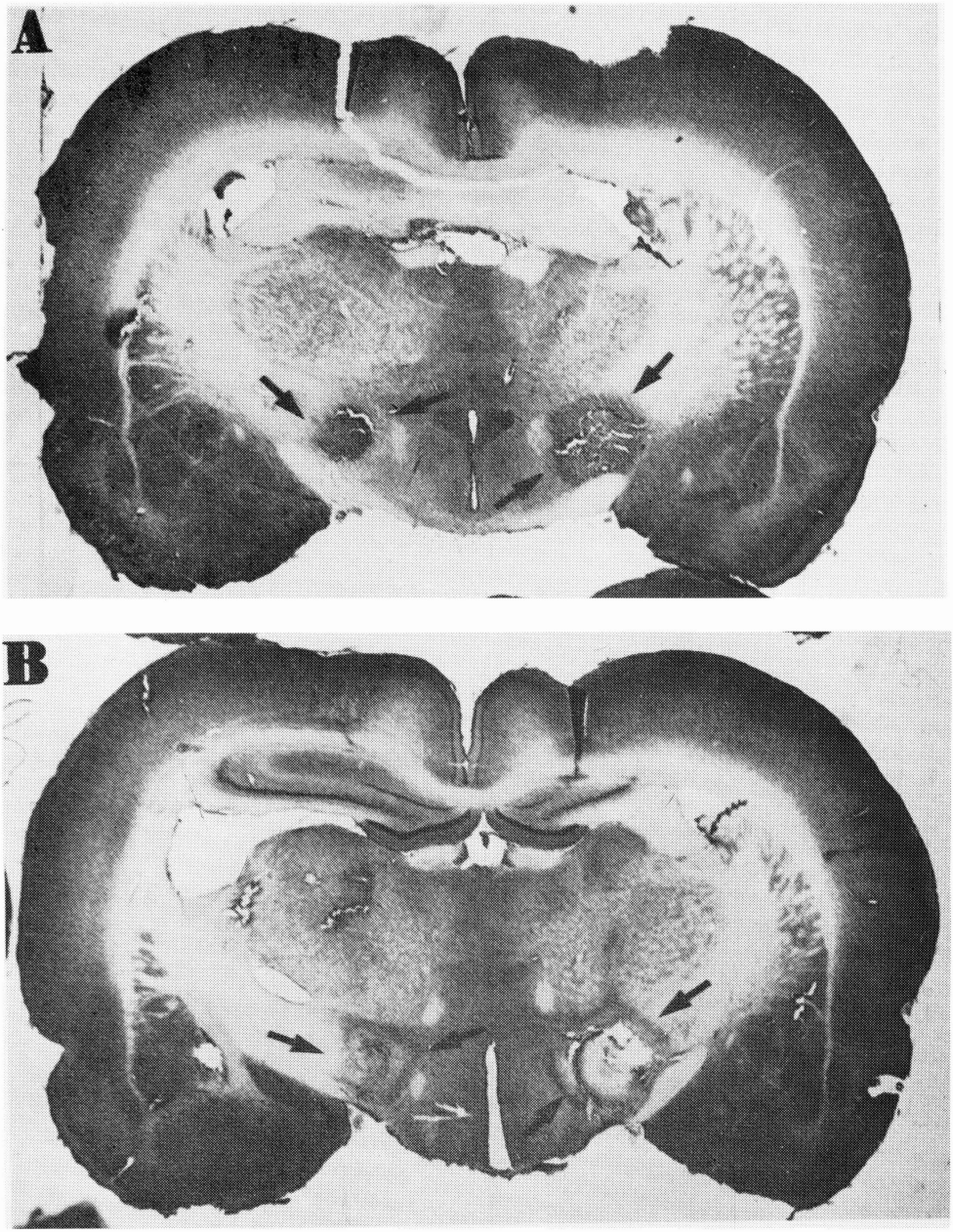
lesions in rats shoving only a slight decrease in food intake were not distinctly different from those of undisturbed animals (Table III).

The animals showing 2 day or longer aphagia usually bore lesions of the tuberal part of LH (level of the ventro-medial nucleus). In cases of 2 - 8 day aphagia (followed by 4 - 14 day hypophagia) lesions were bilateral and ranged from medium-sized to large (Table II). Frequently they were accompanied by large damage to the posterior LH, sometimes encroaching upon the anterior midbrain. This group also contained the rats with lesions placed exclusively within the midbrain, where they involved parts of substantia nigra, ventral tegmental area, medial lemniscus and cerebral peduncle. Damage to the nigro-striatal system in the 2 - 8 day aphagia group was usually bilateral and large, frequently dissecting the fibers of the nigro-striatal bundle. In some animals, however, only unilateral or small lesions of this system were found. Mezo-limbic pathways were also disrupted, although to a different degree, in all the animals of this group.

In the animals suffering from the longest aphagia (9 - 12 days), bilateral lesions of the LH region were very large (e.g. Fig. 1), and in the majority of cases they destroyed LH tissue at all levels of its rostro-caudal axis. In the latero-medial dimention, destruction was frequently total or almost total. The lesions also involved large parts of the neighboring subthalamic structures. There was no damage to the midbrain in this group of animals. The nigro-striatal bundle as well as the mezo-limbic pathways were totally disrupted in the vast majority of animals.

On the basis of correlation analysis, the highest relation ($r = 0.52$; $p \leqslant 0.001$) was found between the presence of feeding disturbances and the presence of lesion within the tuberal part of LH. Analogous values for the posterior LH and the entire LH (nondivided) were 0.22 and 0.36 respectively. The highest probability of receiving at least 2 day aphagia, was connected with symmetrical LH lesions, at the level of the ventro-medial nucleus, which damaged bilaterally not less than 50% of the volume of the tissue. Duration of aphagia was positively correlated ($r = 0.67$; $p \leqslant 0.001$) with the size of the damage to this region. It is interesting to note that the lesions, even large, placed in the anterior or posterior LH never gave an abolition of the feeding behavior as profound as those of the tuberal part. Anterior LH lesions were found only in few animals, posterior LH damage was frequent in disturbed as well as in undisturbed rats. The value of the correlation coefficient between the size of the posterior LH damage and duration of aphagia was only 0.35.

Relatively high correlation ($r = 0.64$; $p \leqslant 0.001$) was found between the size of the damage to the whole LH (nondivided) and a number of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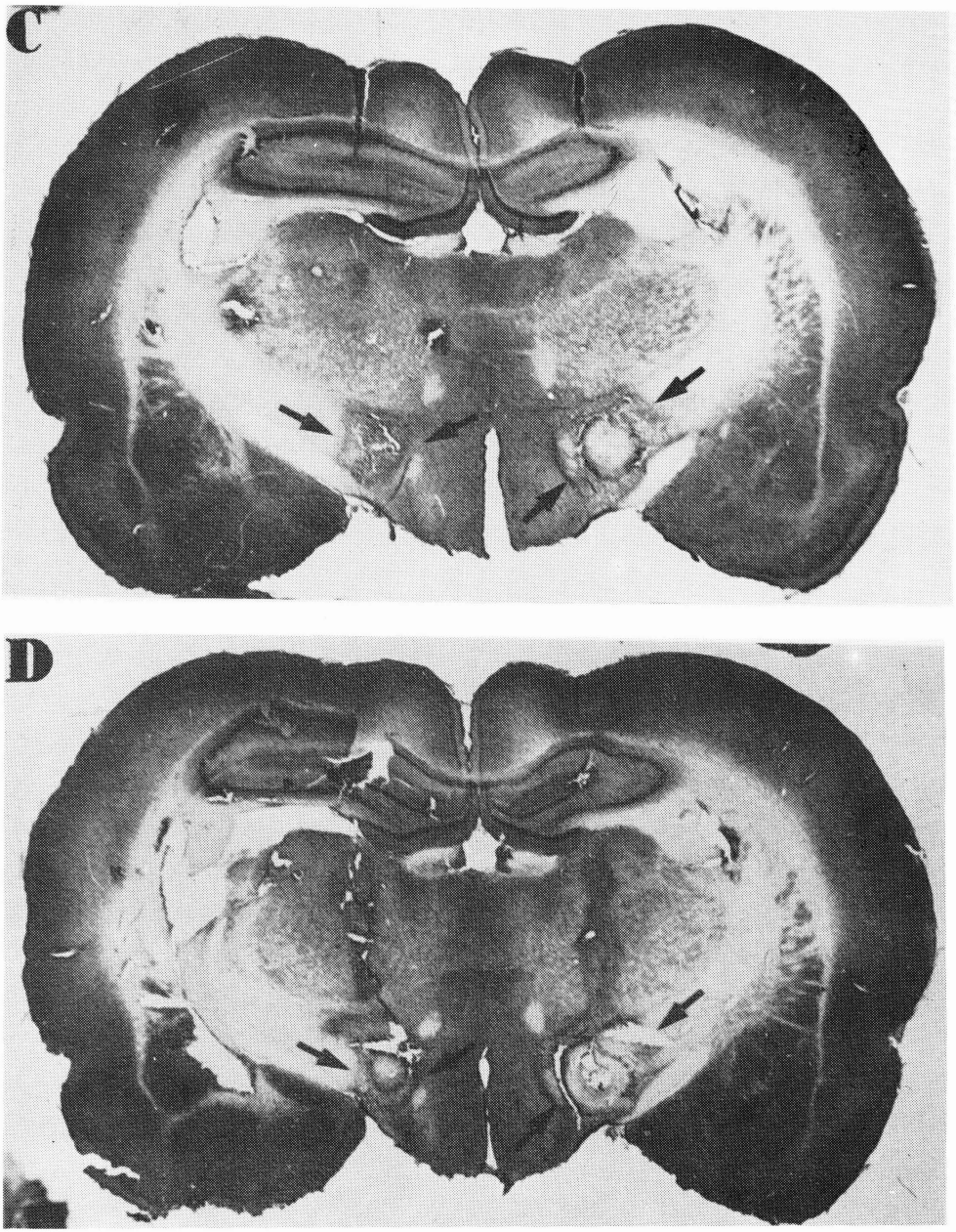


Fig. 1. Photographs of the coronal sections through anterior (A) and tuberal (B–D) LH in example rat (Ko 512) belonging to the group with the largest LH lesions (damage to the posterior LH is not shown). Boundaries of the lesions are marked by arrows.

days of aphagia. This reflects the fact that the longest aphagia (exceeding 8 days) accompanied, in the majority of cases, the lesions involving all three levels of the antero-posterior axis of LH.

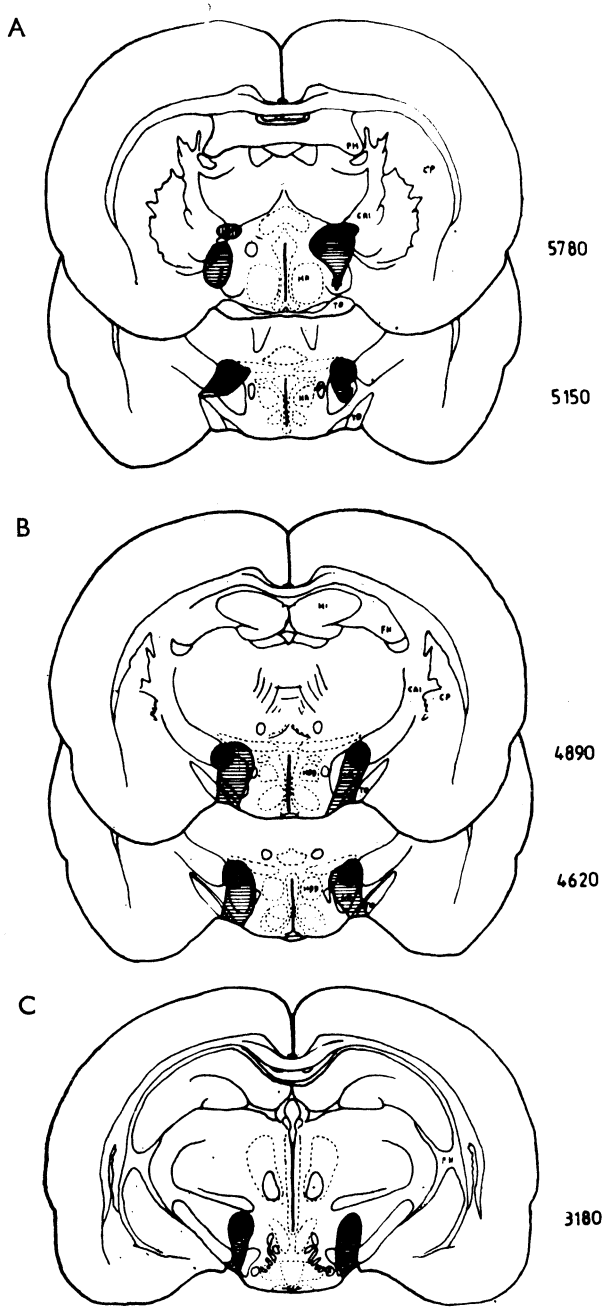
The nigro-striatal system was damaged in 93.8% of disturbed and in 75.0% of undisturbed rats. The correlation coefficient between the presence of the lesion within this system and the presence of feeding disturbances was only 0.37. Higher correlation ($r = 0.60$; $p \leqslant 0.001$) was found between the size of the lesion to the nigro-striatal bundle and the duration of aphagia. The bundle was totally, or almost totally disrupted at the diencephalic level in rats with longest lasting aphagia (Table II). It should be pointed out, however that total or almost total disruption of the bundle either anteriorly or posteriorly to the tuberal region gave only small, if any, reduction of food intake (e.g. rats EEG 44 on Table II and Ko 525 on Table III). This finding may question the importance of the ascending dopaminergic pathways for the feeding behavior. Nevertheless, the lesions localized mainly within the midbrain, which damaged remarkable parts of the substantia nigra, pars compacta and disrupted about 50% of the nigro-striatal bundle at its most posterior level, produced relatively long lasting aphagia (e.g. rat IP 105, Table II). Four types of disruption of the nigro-striatal bundle found in the experimental rats are shown in Fig. 2.

The mezo-limbic pathways (mainly ventral and intermediate paths according to the nomenclature of Simon et al. (27)) were lesioned in 96.9% of disturbed, and in 79.2% of undisturbed rats. The correlation between the size of the mezo-limbic pathways damage and the duration of aphagia was 0.50 ($p \leqslant 0.001$). Since the mezo-limbic fibers are spread almost throughout the whole LH area, they are disrupted in a close proportion to the size of LH damage.

No marked correlation was found between the duration of aphagia and the size of lesions of the zona incerta ($r = 0.16$), fields of Forel ($r = 0.35$), internal capsula ($r = 0.35$), medial lemniscus ($r = 0.03$) and the ventral tegmental area ($r = -0.07$).

Analysis of damage in undisturbed animals. Out of 52 rats in which feeding disturbances did not exceed those in the control group, in 10 histological verification revealed no brain damage (probably because of the failure of lesion maker). The results obtained from these rats provide the support for our criterion of distingushing the effects of brain damage from the unspecific disturbances resulting from the surgery.

Out of the remaining 48 animals, 28 bore lesions within LH, in 20-lesions were localized entirely outside LH, and involved the prosencephalic structures (2 rats), zona incerta at the level of the midbrain (6 rats), the midbrain area comprising the substantia nigra, ventral tegmental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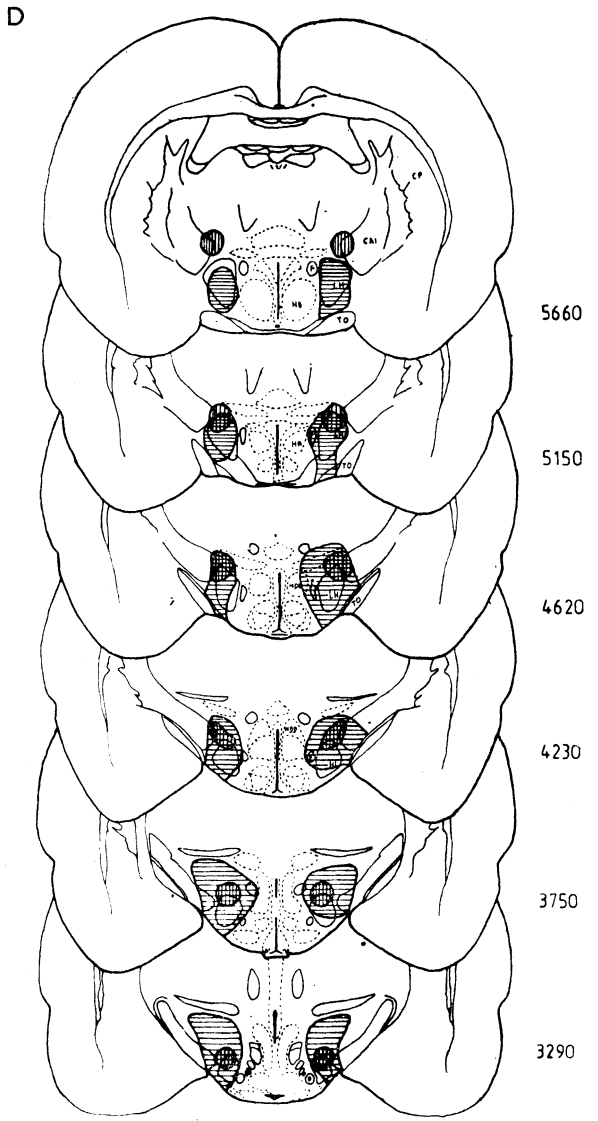

Fig. 2. Localization of example lesions (rats EEG 44, Ko 522, Ko 525 and Ko 512) in relation to the course of the nigro-striatal bundle at the level of the anterior (A), tuberal (B), posterior (C) and all three levels (D) of the antero-posterior axis of LH. Horizontally striped bars, localization of the lesion; vertically striped bars, the course of the nigro-striatal bundle; chequered bars, superposition of the lesion and the course of the nigro-striatal bundle.

area, zona incerta, medial lemniscus and cerebral peduncle (10 rats) and thalamic nuclei (2 rats).

LH damage could have been localized at any of the main three levels of its antero-posterior axis (anterior, tuberal or posterior) and could involve one or two of these levels. Most frequently the lesions of different size (destroying from only a small part up to almost total area in the medio-lateral dimension) were placed within the posterior LH. In the majority of cases they were bilateral. Damage to the tuberal part of LH, if present, was either unilateral or bilaterally small (not exceeding 50% of the medio-lateral dimension of the area). Two rats had lesions within the anterior LH, in one of them the destruction involved up to 75% of the area.

The nigro-strialal system was lesioned in 75% of the rats from this group (in 58.3% bilaterally). In the majority of cases the damage was situated at the level of caudal LH, or the anterior midbrain. The size of the disruption of the nigro-striatal bundle ranged from a small one up to 100% of the area occupied by its fibers. The mezo-limbic pathways were damaged in the majority of animals although not extensively. Table III shows anatomical verification of 17 undisturbed animals representing the above described types of brain damage. The frequency of lesions for the particular structures was specified in Table I.

Daily water intake. Decrease in water ingestion exceeding unspecific effects of the surgery, was found in 102 rats from this group. In the remaining 37 animals adipsia did not occur and hypodipsia did not last longer than 4 days. Out of the undisturbed animals, in 10 rats the histological verification did not reveal any brain damage, and 27 rats were found to bear the lesion. For reasons mentioned earlier, histological analysis was performed on 85 out of the total number of subjects showing drinking impairments.

Depending on the parameters of the lesions, various degrees of disturbances in water intake were found in different animals. 24 rats were only hypodipsic (for 5 - 22 days), in 18 others — one day adipsia was followed by a period of hypodipsia. In 43 rats 2 - 14 day total adipsia occured. Similarly to feeding disturbances, no correlation was found between the duration of adipsia and duration of hypodipsia ($r = 0.06$).

Analysis of lesions in disturbed animals and correlation between adipsia and the brain damage. Anatomical localization of lesions as well as the correlation between the degree of damage to particular structures and the duration of drinking deficits, were generally similar to relations described for impairments in food intake. Drinking behavior, however, appeared to be more sensitive to brain damage and the decrease in water ingestion was also found in rats undisturbed in their daily food intake.

TABLE IV

Frequency of damage to the particular diencephalic and mesencephalic structures in rats showing impairments in daily water intake (+) and in animals undisturbed (—) after LH lesions

Brain region	Impairments in daily water intake	Number (and %) of animals bearing damage to the given area			Number (and %) of animals without damage to the given area
		total	including lesions		
			bilateral	unilateral	
Lateral hypothalamus (anterior)	+	11 (12.9)	9 (10.6)	2 (2.3)	74 (87.1)
	—	4 (14.8)	4 (14.8)	0	23 (85.2)
Lateral hypothalamus (tuberal)	+	49 (57.6)	36 (42.4)	13 (15.2)	36 (42.4)
	—	5 (18.5)	4 (14.8)	1 (3.7)	22 (81.5)
Lateral hypothalamus (posterior)	+	59 (69.4)	48 (56.4)	11 (13.0)	26 (30.6)
	—	8 (29.6)	6 (22.2)	2 (7.4)	19 (70.4)
Zona incerta	+	70 (82.4)	44 (51.8)	26 (30.6)	15 (17.6)
	—	18 (66.7)	9 (33.3)	9 (33.3)	9 (33.3)
Fields of Forel	+	48 (56.5)	31 (36.5)	17 (20.0)	37 (43.5)
	—	9 (33.3)	7 (25.9)	2 (7.4)	18 (66.7)
Subthalamic nucleus	+	39 (45.9)	19 (22.3)	20 (23.6)	46 (54.1)
	—	3 (11.1)	0	3 (11.1)	24 (88.9)
Capsula interna and/or cerebral peduncle	+	43 (50.6)	20 (23.6)	23 (27.0)	42 (49.4)
	—	10 (37.0)	5 (18.5)	5 (18.5)	17 (63.0)
Lemniscus medialis	+	40 (47.0)	27 (31.8)	13 (15.2)	45 (53.0)
	—	4 (14.8)	3 (11.1)	1 (3.7)	23 (85.2)
Substantia nigra	+	23 (27.0)	13 (15.2)	10 (11.8)	62 (73.0)
	—	5 (18.5)	2 (7.4)	3 (11.1)	22 (81.5)
Nigro-striatal system: pars compacta of the substantia nigra and N-S bundle	+	82 (96.5)	65 (76.5)	17 (20.0)	3 (3.5)
	—	14 (51.8)	13 (48.1)	1 (3.7)	13 (48.2)
Ventral tegmental area	+	23 (27.0)	16 (18.8)	7 (8.2)	62 (73.0)
	—	3 (11.1)	3 (11.1)	0	24 (88.9)
Mezo-limbic system: VTA, ventral and medial mezo-limbic pathways	+	83 (97.7)	75 (88.2)	8 (9.5)	2 (2.3)
	—	18 (66.7)	15 (55.6)	3 (11.1)	9 (33.3)

Out of 85 rats which showed drinking deficits 70 (82.4%) had bilateral or unilateral lesions within LH. In 15 rats (17.6%) lesions were localized entirely outside LH (13 within the midbrain; 2 — in the subthalamic region). Similarly to feeding results, other most frequently damaged brain areas were: the zona incerta (82.4% of cases), the nigro-striatal

system (96.5% of cases), and the mezo-limbic pathways (97.7% of cases). Table IV shows the frequency of damage to the particular brain structures.

Table II presents anatomical verification of the example rats chosen in order to show the main types of brain damage together with the duration of drinking deficits resulting from the lesions. The highest correlation between the duration of adipsia and the size of the lesion was found for the tuberal region of LH ($r = 0.60$; $p \leqslant 0.001$) and for the entire LH, regardless of its subdivisions ($r = 0.59$; $p \leqslant 0.001$). Analogous value for the nigro-striatal bundle was 0.50 ($p \leqslant 0.001$); for the mezo-limbic pathways — 0.45 ($p \leqslant 0.001$) and for the subthalamic nucleus — 0.40 ($p \leqslant 0.001$). Little or no relations were found between the number of days of adipsia and the size of damage to the posterior LH ($r = 0.35$), fields of Forel ($r = 0.34$), internal capsula ($r = 0.35$), zona incerta ($r = 0.19$), substantia nigra ($r = -0.03$), and the ventral tegmental area ($r = 0.10$).

Moderate drinking disturbances (adipsia 0 - 2 days, hypodipsia 5 - 15 days) were found in 26 rats, unchanged with respect to their daily food consumption. 18 of them had lesions either at the junction of the posterior LH and the anterior midbrain, usually with an involvement of the zona incerta tissue, or entirely within the ventro-lateral midbrain. In 8 subjects small lesions were spread along different levels of LH.

Analysis of damage in undisturbed animals. In 14 out of 27 undisturbed animals bearing the brain damage, the lesions involved LH area, and in the remaining 13, the lesions were entirely outside the hypothalamus: in the prosencephalic structures (2), midbrain (2), and the posterior zona incerta (7). The frequency of damage to the particular di- and mesencephalic structures in these animals is shown in Table IV.

DISCUSSION

The mapping study presented here strongly suggest that the tuberal part of the lateral hypothalamus is the most probable morphological substratum for feeding and drinking deficits after electrolytic lesions of LH. Out of all the analyzed structures, the highest correlation was found between the presence and intensity of aphagia and adipsia and the presence and size of the lesions within this part of LH. In the majority of cases two day or longer aphagia and adipsia indicated that at least 50% of the LH tissue at the level of the ventro-medial nucleus was destroyed bilaterally. Aphagia and/or adipsia lasting longer than 8 days usually followed huge lesions involving all antero-posterior subdivisions of LH, as well as a substantial parts of adjacent subthalamic area. The damage

to LH at the level of the anterior or posterior hypothalamic nuclei gave 0 - 1 day aphagia and 0 - 3 day adipsia, followed by a period of hypophagia and hypodipsia. These results constitute an exact replication of the findings published in 1961 by Morgane (21).

The most interesting part of our data concerns the relation between ingestive disturbances and the nigro-striatal system. No marked correlation was found between the appearance of feeding and drinking deficits after the lesion, and the presence of damage within the nigro-striatal system. The area covered by the nigro-striatal elements was frequently damaged in both disturbed and nondisturbed animals. Relatively high correlation between the duration of aphagia and adipsia and the size of nigro-striatal sytem lesions reflects the fact that the nigro-striatal bundle was destroyed in close proportion to the size of LH damage. The same concerns the mezo-limbic pathways. Only a slight decrease in food and water intake was found in rats with total or almost total disruption of the nigro-striatal bundle at the levels anterior or posterior to the tuberal LH. This finding corresponds to the data reported by Hodge and Butcher (10), who did not observe aphagia and adipsia after complete and precise radiofrequency lesions of the pars compacta of the substantia nigra. Similar results were reported by Konopacki (12). Also Dourish (5) in his rewiev of the role of dopamine in thirst and drinking says that although ascending dopaminergic pathways may play a part in motor responses associated with drinking behavior, the interruption of the nigro-striatal axons is not a primary cause of the drinking deficits associated with extensive electrolytic lesions of LH. There is, however, a bulk of pharmacological evidence (4, 6, 14 - 16, 25, 26, 35, 37, 48, 49) pointing out to an essential role of nigral dopaminergic neurons in the etiology of LH aphagia and adipsia. The apparent discrepancy between the results obtained by means of unspecific lesions and those found in 6-hydroxydopamine (6-OHDA) studies is difficult to resolve at present. Hodge and Butcher (10) suggest the involvement of an unspecific damage component in every intranigral 6-OHDA injection. This does not, however, explain ingestive deficits following 6-OHDA injections into the cerebral ventricles (4, 37, 48, 49). It does not seem likely that the nigro-striatal bundle is the only one, or even decisive morphological element responsible for LH ingestive syndrome. LH neuronal somata seem to be by far more specifically related to food (27, 32) and water (11, 28, 38, 39) intake. However, lateral hypothalamic lesions which do not involve the nigro-striatal fibers e.g. by means of kainic (9, 36, 46) or ibotenic (47) acids or electrocoagulation of the mid-lateral part of the hypothalamus (21) never give as profound and long-lasting disturbances as those involving also the course of the nigro-striatal bundle. Our data do not indicate any serious role of the nigro-striatal damage in the etiology of LH ingestive

impairments, nevertheless it is hard to ignore extensive pharmacological literature concerning dopamine depletion-induced aphagia and adipsia. It seems that the real meaning of these pharmacological data is still to be cleared up.

Morphological substrate of drinking deficits closely paralleled those of feeding disturbances. High correlation ($r = 0.85$) was also found between the duration of aphagia and adipsia. However, drinking appeared to be more sensitive to brain damage and moderate decline of water intake was found in rats undisturbed in their daily food ingestion.

Close relationship between food and water intake have been shown in many experiments. Usually, manipulations affecting the consumption of one are followed by a parallel change in the intake of the other. This also concerns central manipulations. It is well established that there is extensive overlap between central feeding and drinking elements, particularly within he lateral hypothalamic area (e.g. see 22). However, Montemurro and Stevenson (19) were able to isolate regions where selective reduction of water intake was obtained without a simultaneous change in food consumption by means of small lesions within the tuberal LH. We found a similar area in the posterior LH and the anterior midbrain. Feeding and drinking effects of the brain damage can also be differentiated at the level of the preoptic area (1, 21), the zona incerta (45) and some limbic structures (18). Review of data concerning ingestive effects of electrical brain stimulation (18, 21) also indicate that food intake and water intake are separable anatomically.

Quantitative analysis of our results consisted in correlating the presence and intensity of ingestive impairments with the presence and the size of the damage to the particular brain structures. The obtained values of a correlation coefficient were not high, even though highly significant. The values assessed as meaningful oscillated around 0.5 - 0.6. The main cause of a relatively low correlation coefficient was a lack of distinctive anatomical differences between unimpaired animals and those showing disturbances of lowest intensity. One may speculate that in the cases of small lesions (smaller than 50% of the total structure volume) of the relevant area, the appearance of a disfunction is determined rather individually than anatomically. Several studies (3, 7, 8, 31, 33) showed that the physiological status of the animal at the time of lesion, greatly influences the course of the LH syndrome. Genetical factors can not be excluded either.

From the clinical point of view, it may be interesting to note that there was no correlation between the duration of the acute stage of LH ingestive syndrome (aphagia and adipsia) and the total duration of disturbances. Similar lack of correlation was found in the case of LH

catalepsy (unpublished data). Since we did not measure the total duration of the impairments in the most disturbed animals, the above finding concerns at least an incomplete LH damage. Our results indicate that the acute stage of LH syndrome is determined anatomically. One may speculate that the total duration of a dysfunction may also depend on other factors.

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