

## IMPAIRMENT OF SALIVARY REFLEXES AFTER LATERAL HYPOTHALAMIC LESIONS IN DOGS

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**Abstract.** The effects of lateral hypothalamic lesions on salivary conditioned and unconditioned reflexes were investigated on seven dogs. Subsequent to the operation during the period of aphagia and hypophagia a total lack (6 dogs), or a decrease (1 dog) of conditioned salivary reaction was found. Strong diminution of unconditioned reactions and intertrial salivation were observed. At the end of the observation period (7 months) the conditioned and unconditioned salivary reflexes were still diminished reaching levels of 12-54% and 35-75% respectively in comparison with the pre-operative period. The mechanism of the impairment of salivary reactions was discussed.

### INTRODUCTION

Numerous investigations have shown that lesions situated in the lateral hypothalamus impair various alimentary reactions. However, the effects of such lesions on salivary reactions have been little investigated. Most experiments concerning the role of the hypothalamus in regulation of food intake have been performed on cats and rats, in which cases the recording of salivation is much more difficult than on dogs. Even for dogs, however, we were able to find only one investigation on salivary reactions in relation to the hypothalamus (Chereshnev 1960).

Our recent series of experiments has dealt with the role of the hypothalamus on alimentary behavior of dogs (Rożkowska 1969, Fonberg and Rożkowska 1968, Fonberg 1969, Rożkowska and Fonberg 1970, 1971). We found that lesions of lateral hypothalamus produced aphagia and adipisia. In addition, the dogs seemed to become atonic and lacking in drive. Their instrumental reactions were deeply impaired or completely lost. These symptoms may indicate that lateral hypothalamic lesions impair mainly

the hunger drive, and in consequence impair the instrumental and other voluntary reactions which lead to getting and consuming food. On the other hand the lateral hypothalamic lesions also impaired such involuntary reactions as stomach contractions (Glavcheva et al. 1970) and produced vomiting (Fonberg and Rożkowska 1968, Rożkowska 1969). This indicates that the lateral hypothalamus is involved in various alimentary functions, instrumental and consummatory, voluntary and autonomic. It was therefore considered of interest to see whether salivary reactions would also be impaired by lateral hypothalamic lesions and whether this impairment would concern both conditioned and unconditioned salivation.

#### MATERIAL AND EXPERIMENTAL PROCEDURE

Experiments were performed on 7 naive male mongrel dogs, weighing from 9 to 12.5 kg. All these dogs had a chronic gland fistula performed by Sołtysik and Zbrożyna method (1957). The fistula was made on the right side. Saliva from the Stensen's duct fistula was collected into a capsule attached to the dog's cheek. A tube running from the collecting cup to the recording equipment was filled with water so that, as saliva was secreted, drops of water were ejected onto an electric recorder.

After the fistula had been made, the experiments were started in a conditioned reflex chamber. The experiments were run on dogs unfed for ca. 20 hr. At first the dogs became used to eating from bowls moving automatically in the feeder placed within a distance of 18 cm from the mouth and also to the saliva recording procedure, especially to fixing a capsule onto the cheek by means of specially prepared sealing wax (Mendeleyev type). The preparatory period lasted about 2-4 days and then, by the classical method, the training for the conditioned alimentary reflex was started. Each dog received 50 g of food in a bowl before the experiment. The conditioned stimulus was a sound from a generator of the value 1,000 cycle/sec, reinforced by food (bread powder mixed with boiled minced meat — 50 g). The experiments were carried out daily and each session consisted of six trials, intertrial intervals lasting 3-4 min. Initially the conditioned stimulus slightly preceded the intake of food (2 sec), afterwards progressively from day to day its isolated period was prolonged from 2 to 20 sec. The overlapping CS-US was 10 sec making the total duration of CS 30 sec. It was estimated that the conditioned reflex was established if within 10 consecutive experiments the amount of saliva secreted on CS remained stable i.e. about 15-18 drops.

The food intake was measured. The dogs were allowed to eat ad lib. once a day at 2 PM in the doghouse during 10 days prior to and 20 days following the operation, then again during a 20 day period 1 month later.

The dogs were weighed every fourth day: five times before the operation, five times after the operation and again five times after a month interval. Also the body temperature was measured (rectally), for 10 days both before and after the operation.

**Surgery.** When the whole experimental training was completed and the conditioned reflex established, electrocoagulation of the lateral hypothalamus was performed. Surgical procedure were done in aseptic conditions under Nembutal anesthesia (35 mg/kg). The electrodes were stainless steel needles 0.4 mm in tip. Electrodes were placed stereotaxically and aimed at the lateral part of the hypothalamus, according to the coordinates based on the stereotaxic atlas (Lim et al. 1960). Direct current 3 ma of 1 min duration was used. Animals were under observation for 6–8 months.

**Histology.** After the experiments were accomplished, the dogs were sacrificed and their brains perfused with 10% formalin. Three brains were embedded in paraffin and 20  $\mu$  thick slices were stained alternately by means of the Klüver and Nissl methods. The remaining brain were frozen and 40  $\mu$  thick slices were stained by means of the Klüver method.

## RESULTS

**Food and water intake.** After the operation aphagia and adipsia were observed in all dogs. Total aphagia lasted 3–17 days (Fig. 1) and adipsia 2–4 weeks. During this period the dogs were kept alive by means of feeding through a stomach tube. Three times a day half-liquid food was injected: twice a day in the form of broth with rubbed cereal and once a day a mixture prepared from milk, egg yolk, sugar and vitamins.

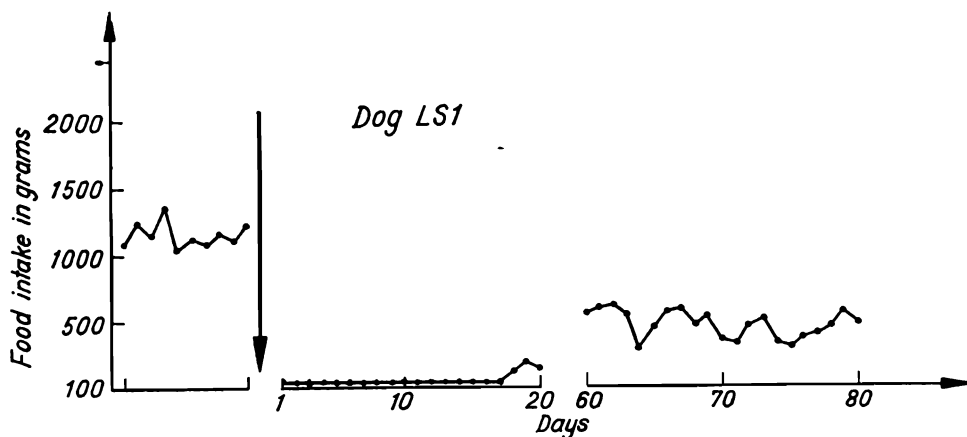


Fig. 1. Daily food intake in a representative dog (LS1).

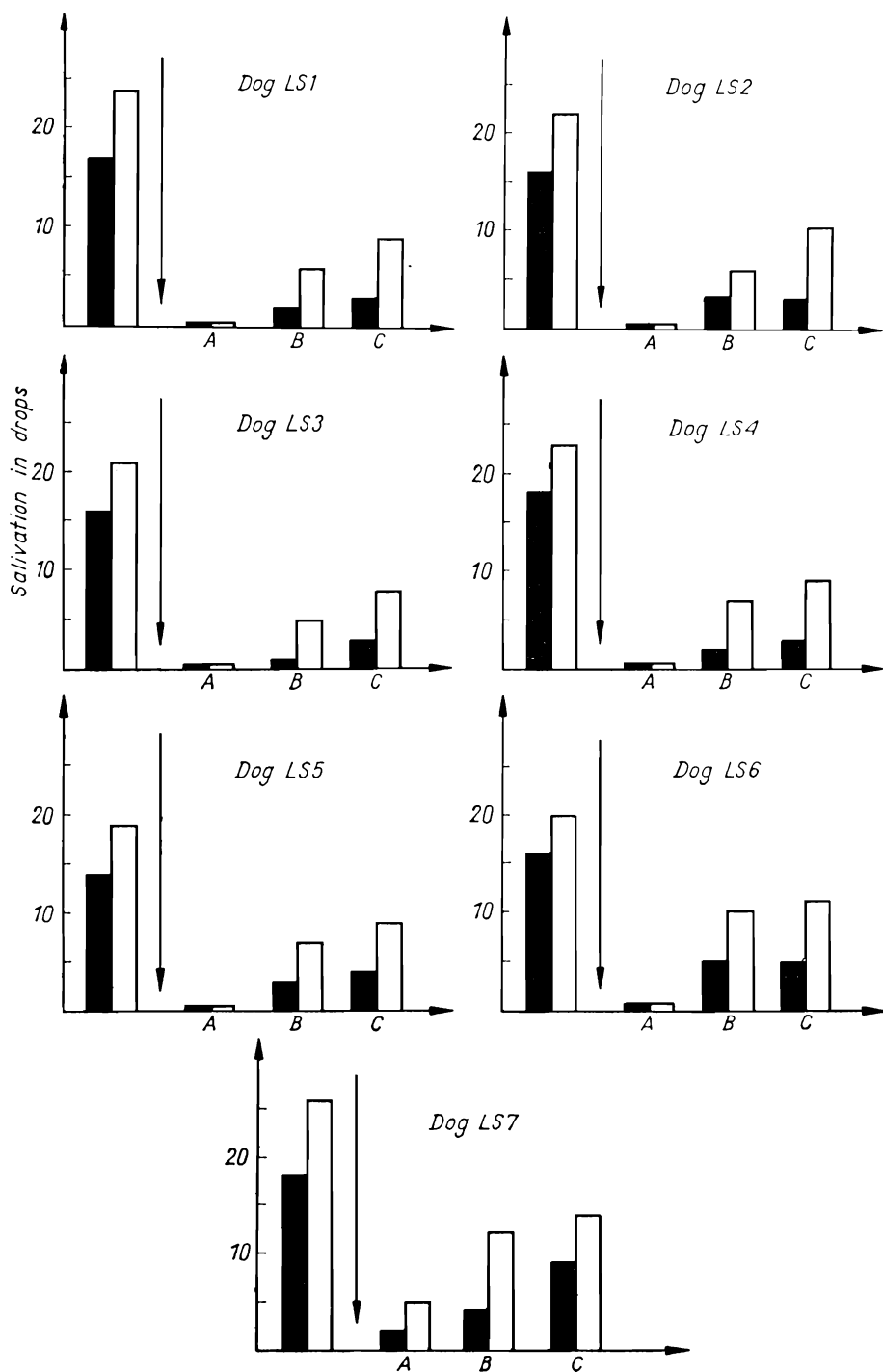
After almost every meal the dogs vomited, in most cases within 10 or 20 min after food intake. Especially persistent and frequent vomiting was observed in the LS1 and LS3 dogs and therefore in order to prevent dehydration these dogs were injected a physiological salt solution (200–250 ml) and 5% glucose (60 ml) with vitamins C and B. Gradually the dogs started to feed themselves. At first they were encouraged in many ways to eat i.e. the bowls were moved towards them, their heads were bent down to the bowls or small bits of food were put into their mouths, more attractive food was served, and so on. It occurred that the dogs definitely refused to be fed, turned away their heads and rejected spooned food (Fig. 2). In spite of all these procedures they ate very little about 50–100 g, so once a day they had to be fed by stomach tube. In their first attempts at eating the movements of jaws and tongue were clumsy; they splashed the food around and often choked. For about 3 weeks they showed preference for sweet and semi-liquid food and refused to eat raw meat. They drank milk in small quantities but no water. Hypophagia lasted for a long time and towards the end of the observation period (6–8 months) the majority of the operated dogs ate half of their regular portions. In the first weeks of hypophagia vomiting occurred frequently, in particular in dogs LS1, LS2, LS3, and LS4, but later occurred only sporadically.

*Temperature and body weight.* During the period of aphagia and in the first weeks of hypophagia the body weight decreased ca. 1–3.3 kg. In the later period the dogs gradually gained weight, but none reached the pre-operative weight. Within the first 10 days after the electrocoagulation the body temperature was slightly lowered (ca. 1°C).

*General behavior.* A marked decrease of general activity was observed in all dogs. They walked slowly, looked sad, apathetic and somnolent. It was difficult to keep them in cleanliness, because they often lay down in their excrement and vomit. In five dogs (LS1, LS2, LS3, LS5 and LS7) during the first weeks after the operation, cataleptic-like postures often occurred, i.e. for an hour or more they would stand or sit in uncomfortable positions, would freeze in some strange position with a lifted paw, with a twisted trunk and head, and so on. All the dogs showed symptoms of negativism (Fig. 2) and lost their former friendliness towards people as described in detail in previous papers on an other group of dogs with

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Fig. 3. The effect of lateral hypothalamic lesion on conditioned salivary reflex (black blocks) and unconditioned salivary reflex (white blocks). Blocks represent mean value of saliva secretion of 10 experiments before the operation; 10 first experiments after the operation (A); 10 experiments after 2 months interval (B); then of 10 experiments after 4 months (C). Arrows indicate the day of operation. Salivation for 20 sec after presentation of food is regarded as unconditioned reflex.



lateral hypothalamic lesion (Rożkowska 1969, Fonberg 1969, Rożkowska and Fonberg 1970).

*Secretion of saliva on unconditioned and conditioned alimentary stimulus.* The experiments were resumed on the third day after the operation and carried on every third day during the period of aphagia. Regular experimental sessions started when the dogs began to eat spontaneously. In the period of aphagia and hypophagia in six dogs a total lack of conditioned salivary reaction was found lasting 18–49 days, and in addition secretion of saliva on the unconditioned stimulus was decreased to 15–30% in comparison with the pre-operative value (Fig. 3). In one dog (LS7) during the first 20 days after the operation, only a decrease of saliva secretion on the conditioned and unconditioned stimulus was observed

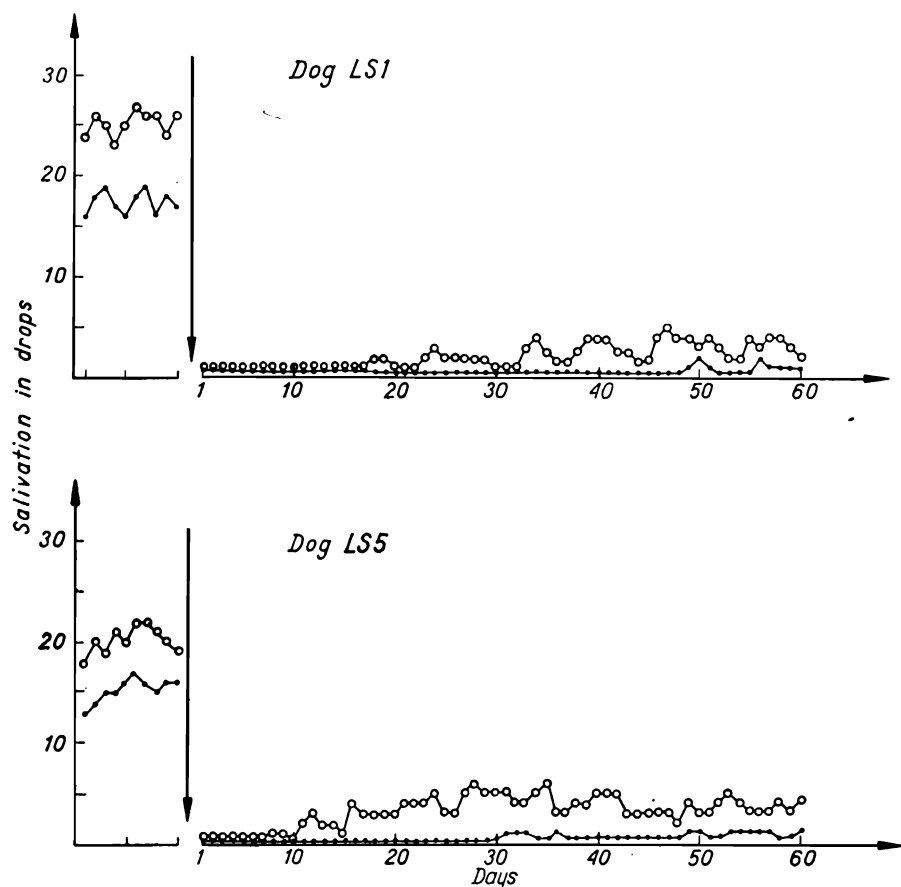


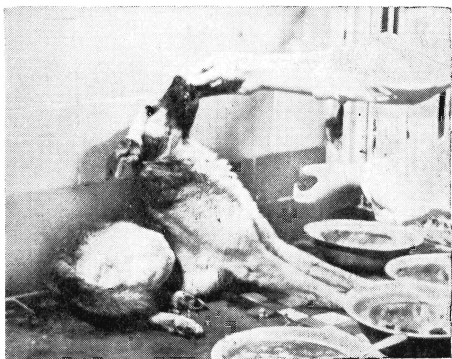
Fig. 4. Secretion of saliva on CS (line with black circles) and on US (line with open circles) in dogs LS1 and LS5. Arrows indicate the day of operation.

*Dog LS1*

*A*



*B*



*C*



*Dog LS5*



Fig. 2. Post-operative behavior of the dogs LS1 and LS5. A, lack of interest in various kinds of food; B, withdrawal from feeding and rejection of food; C, negativism.

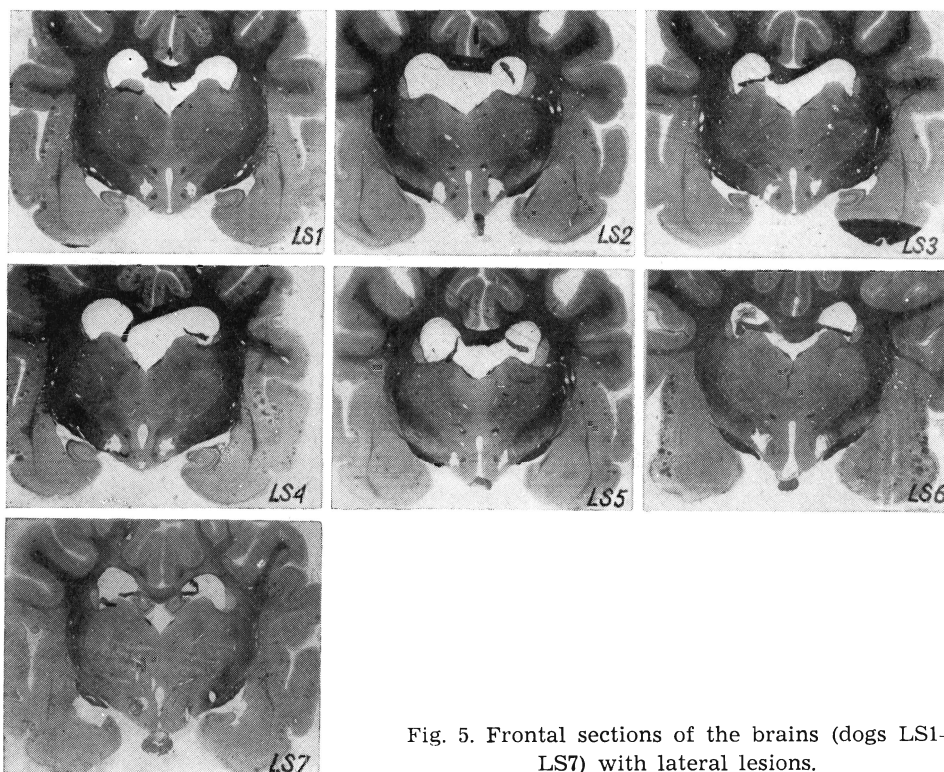


Fig. 5. Frontal sections of the brains (dogs LS1-LS7) with lateral lesions.



(to 10 and 25% of the pre-operative value respectively). Intertrial salivation in all dogs decreased very strongly i.e., to 15–35% in comparison with the pre-operative period. After 7 months 150 experiments (900 trials) had been completed. Percentage of the amount of saliva as responses to the conditioned stimulus in particular dogs LS1–LS7 ranged from 12 to 54%, and the percentage of amount of saliva secreted during 20 sec of food intake (on US) ranged from 35 to 75%.

Figure 4 shows that secretion of saliva first appears after the operation on unconditioned stimulus and then several days later (in one case — dog LS1 — after 49 days) on conditioned stimulus.

For statistical elaboration of the results obtained Wilcoxon's test was used. In all dogs we compared the mean absolute number of drops of saliva secreted during the isolated period of the conditioned stimulus (20 sec) from last 10 experiments before the operation with the 10 days in the post-operative period and then with the corresponding period after the lapse of 2 and 4 months. Each of these three periods differed in comparison with the pre-operative period at a significant level ( $p < 0.02$ ). In the same manner we compared the mean absolute number of drops of saliva secreted within 20 sec of food intake. Each of the above mentioned periods differed significantly from the pre-operative state ( $p < 0.05$ ). Also we compared in all dogs the mean absolute quantities of food intake from the 10 days pre-operative and 20 days post-operative and with a 10 day period after a month interval. The observed changes in food intake proved to be statistically significant ( $p < 0.02$ ).

*Anatomical verification.* In all the dogs the lateral nucleus of the hypothalamus was damaged bilaterally. As well, the lesion damaged partially Forel's area  $H_2$  and zona incerta. In the lateral plane the lesion extended from the fornix region slightly beneath it and farther laterally, damaging the infero-ventromedial part of the internal capsule and partially the nucleus entopeduncularis (in dogs LS2, LS3, LS4 and LS7). In dogs LS2, LS3, LS4, LS6 and LS7 the fibers of ansa lenticularis were partially destroyed. (Figure 5 shows the lesions in all dogs.)

## DISCUSSION

The results of our experiments clearly show that lesions situated in the lateral hypothalamus, besides all other symptoms described previously as the "lateral hypothalamic syndrome", produced evident impairment of the salivary reactions as well.

The interesting fact is that impairment of salivation concerns both conditioned and unconditioned reactions and also salivation during the intertrial intervals. This may suggest that it is caused by a general

decrease of function of the salivary mechanisms. The decrease of salivation after lateral hypothalamic lesions was also observed by Hainsworth and Epstein (1966) and Kissileff (1969).

The unconditioned salivary reactions are dependent, according to Wang (1943), on the salivary center in the dorsolateral reticular formation, dorso-medial to the spinal trigeminal nucleus and dorsal to and at the level of the facial nucleus. The rostral portion of this area supplies the submaxillary glands while the caudal part supplies the parotis. Through this area the unconditioned salivary reflex arc passes. Although the direct neural connections between this area and the hypothalamus are not known, this reflex must, however, be under the influence of the lateral hypothalamus as shown by our present experiments. Salivation may be evoked by the stimulation of both the sympathetic and parasympathetic systems, but the abundant outflow of liquid saliva depends on the excitation of parasympathetic system. Therefore the question arises whether the impairment of salivation produced by the lateral hypothalamic lesion is due to the lack of alimentary drive or consummatory reflexes or is simply produced by the impairment of a part of parasympathetic system per se, apart from whether it is an alimentary function or any other.

As shown previously (Glavcheva et al. 1970) such involuntary reactions as stomach contractions, both conditioned and unconditioned, are also impaired by lateral hypothalamic lesions and it is well established that these reactions are also mediated through the parasympathetic system. Yet the damage to the parasympathetic system does not explain other symptoms of the lateral hypothalamic syndrome, i.e., lack of general arousal, lowered body temperature, impairment of instrumental reactions, negativism, and so on. In particular general arousal should rather tend to increase with the impairment of the parasympathetic system and consequent predominance of the sympathetic system.

It is clear that the alimentary drive is lowered by the lateral hypothalamic lesions. Even after recovery from complete aphagia the dogs are still not interested in food, do not try to obtain it, and their instrumental reactions are completely abolished or impaired (Rożkowska 1969, Rożkowska and Fonberg 1970).

According to Konorski's theory (1967) the salivary reactions, both conditioned and unconditioned, are based on the "consummatory" reflexes which are facilitated by hunger drive. Low hunger drive may explain the lack of anticipatory salivation to the conditioned stimulus. The fact that in our experiment the CS salivary reflex was completely abolished, whereas the unconditioned reflex was decreased to 15–30%, strongly suggests that this portion of unconditioned salivation is based on the medullary salivary center. The problem why even fully satiated animals produce

an almost normal salivary unconditioned reaction as shown by Sołtysik (1971) requires further investigations.

Wyrwicka found on goats (W. Wyrwicka, unpublished results (that stimulation of the lateral hypothalamus, in the area where stimulation evoked food consumption in satiated goats, elicits salivation before the instrumental reaction is evoked. This may mean that the lateral hypothalamic "food center" is more primarily connected with salivary reactions than with instrumental and that this area is more connected with food consumption than with hunger drive (Fonberg 1967, 1969).

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