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SALIVARY REACTIONS IN DOGS WITH DORSOMEDIAL AMYGDALAR LESIONS

Jadwiga ŁAGOWSKA and Elżbieta FONBERG

Department of Neurophysiology, Nencki Institute of Experimental Biology
Warsaw, Poland

Abstract. In dogs with chronic parotid fistula, conditioned salivary reactions reinforced by food were established. After bilateral lesions of the dorsomedial part of the amygdaloid complex, the conditioned salivary reactions were greatly diminished. Also the unconditioned salivation decreased. This decrease was greater in dogs which revealed the whole syndrome of amygdalar aphagia, but it was also evident in hypophagic dogs. In some dogs, the disinhibition of the salivation to negatives CS was also observed. Results show that the dorsomedial amygdala, similarly to lateral hypothalamus, is involved in the regulation of salivary reactions.

INTRODUCTION

Alimentary functions are regulated by various brain structures. The best known is the role of the hypothalamus in hunger and satiation mechanisms. However, recently it has been shown by Fonberg (1–5) that bilateral destruction of the dorsomedial amygdala produces effects similar in many aspects to the effects of lateral hypothalamic lesions, i.e., the syndrome of aphagia and adipsia, vomiting, loss of instrumental reactions and general depression. This syndrome is not limited to symptoms dependent on a lowered hunger drive but also involves various alimentary responses, among them the salivary reactions. Rożkowska and Fonberg have shown (13, 14) that classical salivary reflexes, both conditioned and unconditioned, are greatly impaired or even completely abolished in dogs after lateral hypothalamic lesions. Data obtained from stimulation of the amygdala suggest that this structure is also concerned with the secretion of saliva. MacLean and Delgado (12), Wood (21), Ursin and Kaada (19) Sano (15), Kazami (7), Koikegami (9) and Shealy and Peele (16) showed that stimulation of various parts of the amygdaloid complex produced increased salivation. Therefore, it seemed important to study whether or not amygdalar lesions would also change the salivary reflexes in a way similar to that produced by lateral hypothalamic damage. In undertaking such experiments, we hoped that this would help us to determine the role played by the various parts of the alimentary system in the different components of the alimentary act.

METHODS

Experiments were performed on 10 naive mongrel dogs, 2–3 years old and weighing 10–14 kg.

Salivary registration. Before the experiments started, a fistula of the parotid gland was made according to the method of Ganike-Kupalov, as modified by Sołtysik and Zbrożyna (18). The fistula was made unilaterally on the right side and opened on the cheek. The saliva was collected in a capsula attached to the cheek by means of Mendeleev wax. Through a system of tubes the drops of saliva were registered electrically and recorded on a kymograph.

Preoperative training. Training of the conditioned classical salivary reaction started after complete recovery from the fistula operation. During the first 2–6 days the dogs were accustomed to the experimental chamber, harness, feeding from the food-tray and application of the salivary capsule. Then conditioning of classical salivary reactions began. Before each experimental session, the dogs received 50 g of food in the experimental chamber. Each session consisted of eight trials, intertrial intervals being 3–4 min. As the CS^{+}, a tone 1,000 cycle/sec, from a tone generator was used. In the first few sessions, the CS was reinforced by food 2–3 sec after its onset. This period was gradually lengthened until presentation of the CS reached 20 sec. Then food was given, and the CS overlapped food intake for the next 10 sec. In one dog, the food was presented after 10 sec of CS, this dog was used in the pilot experiments, but as the results obtained on him were similar to those of the remaining dogs of group, he was included in this group. The positive salivary reflexes were trained to criterion. As a criterion, we arbitrarily took 10 experimental sessions with conditioned salivation stabilized at a level of 3–15 drops. This criterion of the secretion was different for each dog but not fewer than 3 drops during 10 sec of the positive conditioned stimulus that preceded food reinforcement (US).

Differentiation of positive and inhibitory CSs. When training of a positive conditioned reflex was accomplished, a tone of 500 cycle/sec was introduced as a differentiated stimulus CS^-, not reinforced by food. The duration of the CS^- was 20 sec from the beginning. Starting from this point, each session consisted of eight trials, with four positive and four negative stimuli randomly presented. Training lasted until the animal reached criterion for both kinds of stimuli during 10 successive experimental sessions. The criterion for the positive reaction was the same as during the previous period of the experiment, i.e., at least three drops during 10 sec of the CS^+ preceding reinforcement. The criterion for the salivary reaction during the presentation of the CS^- was always near zero (0–1 drops). As an additional control for the salivary reaction, the amount of salivation during the 20 sec period before and 20 sec after the conditioned stimulus was measured.

For statistical analysis, the salivation from the 11th to the 20th sec of the presentation of CS was calculated, as well as for the next 10 sec when there was an overlapping of CS and UCS, and the following 10 sec of post-stimulus salivation. The first 10 sec of each stimulus presentation were not calculated because of variation in the time required for salivation to begin.

Surgery. The operation was performed stereotaxically under Nembutal anesthesia after the animal reached criterion in differentiation. The dorsomedial part of the amygdala was electrocoagulated by means of a d-c current (2–3 ma, 2–6 v, 1–2 min duration). Steel or wolfram stainless electrodes insulated by enamal except for 1 mm of the tip were used. Stereotaxic coordinates were based on the atlas of Lim, Liu and Moffit (11) with our own corrections for the dimensions of the dogs' skulls.

Postoperative retention tests. The experiments started again on the 3rd and 7th days after the operation (test) and were performed daily beginning 10 days after the operation (retraining). The post-operative observations lasted for about 2 months. Some dogs were then subjected to subsequent operation of the lateral amygdala. The results of this operation will be described in another paper.

Food intake and body weight. In all dogs food intake was measured during the 10-day period before the operation, the 10 days after and 10 days a month later. During these periods the dogs were allowed to eat ad lib. twice a day. The food consisted of cereal mixed with broth. Their body weights were checked every five days.

Histological verification. After the experiments were completed, the dogs were anesthetized and perfused by formalin. Their brains were embedded in paraffin and cut in frontal sections 20 μm thick. Alternate sections were stained by the Klüver and Nissl methods. In some brains, frozen sections were made and stained as above.

RESULTS

Food intake and general behavior. After the operation food intake of the animals was decreased (Fig. 1*A*). Five dogs out of 10 were aphagic

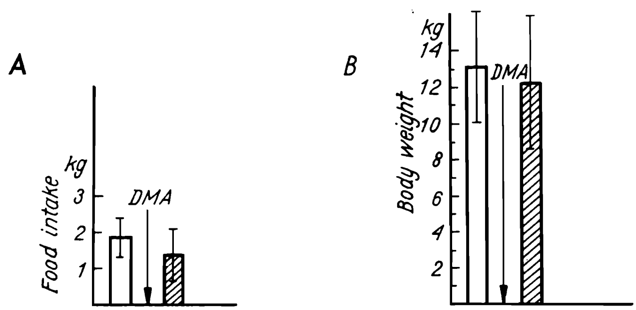

Fig. 1. The effect of dorsomedial amygdalar lesions on food intake (*A*) and body weight (*B*). In *A* columns represent the mean food intake of 10 dogs from the period of 10 days before the operation (white bars) and during the first 10 days after the operation (striped bars). In *B* columns represent mean body weight of 10 dogs before the operation (white bars), and on the 10th day after the operation (striped bars).

or hypophagic, and 5 of them did not show any decrease in food intake (Fig. 1*A*). The most pronounced aphagia (14 days), followed by several weeks of hypophagia, appeared in dog Melange. In Młano, in which total aphagia lasted only 4 days, hypophagia persisted for about a month. Lipień was completely aphagic for 10 days and hypophagic until the end of the observational period after this operation, i.e., 55 days. Kok was hypophagic for 5 days; Piaff was hypophagic for only 2 days.

In the remaining dogs, in spite of no obvious changes in the amount of food intake, disturbances in various patterns of alimentary behavior were observed. Węgorz and Jesiotr vomited after their meals and refused food during the experimental sessions. Szprotka also refused food offered as a reinforcement during the experiments. Mean body weight for all animals was decreased (Fig. 1*B*).

Most of the dogs were changed in their emotionality after the operation. Melange was hypoactive, indifferent, unresponsive to people and apathetic for a few weeks. Młano was also apathetic and indifferent; he lost his friendly attitude toward humans and opposed handling. Lipień was apathetic and hypoactive for several weeks. Kok was depressed and less friendly to the experimenter. Lipień and Szprotka were atonic and apathetic. In addition, Węgorz revealed a tendency to be cataleptic with bizarre postures. Płastuga, Piaff and Węgorz exhibited fear reactions toward unknown persons. The hair of the dogs was thin and dull in appearance, and the skin around the fistula was thin and looked oily. The Mendeleev wax, which was used to fix the ring of the recording apparatus, did not stick to the skin. Several procedures used to clean and adapt the surface of the skin, washing by ethyl alcohol, ether, or scratching the epithelium were ineffective. This symptom greatly interfered with registration of salivation because it was very difficult to keep the apparatus on the skin during the whole experiment.

Salivary reactions. After the operation the following changes in salivary reactions were observed: (i) decrease of salivary reaction to the positive stimuli in nine dogs (Melange, Młano, Kok, Piaff, Płastuga, Lipień, Jesiotr, Węgorz, Szprotka), (ii) slight disinhibition of salivation to the nonreinforced CS in seven dogs (Młano, Kok, Piaff, Skalar, Szprotka, Węgorz, Płastuga). Figure 2 demonstrates the salivary reaction of the dogs before and after dorsomedial amygdala lesions. The conditioned and

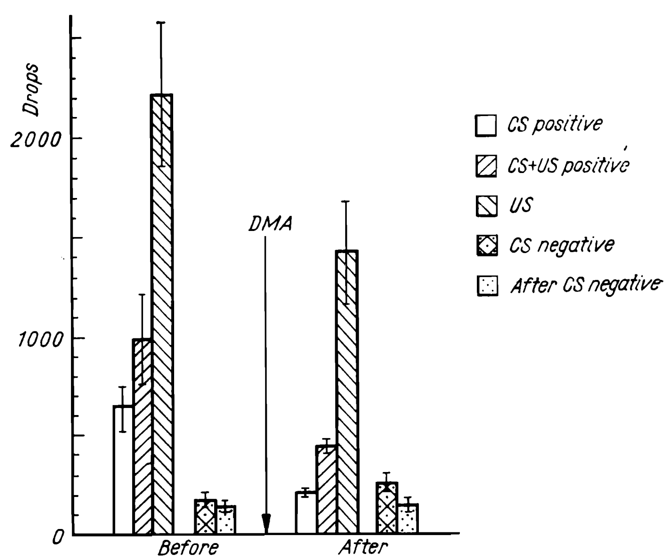

Fig. 2. Mean number of drops of saliva secreted during particular phases of positive and negative stimuli. Columns represent mean value of reactions of 10 dogs during 10 days before and 10 days after the operation.

unconditioned reactions to positive stimuli are clearly diminished although the reactions to negative stimuli are only slightly increased just after the operation. Figure 3 illustrates the salivary reactions of the dog Melange before and after the operation. The results of each individual dog show that the general trend of reactions was the same in all operated dogs, except for one, Skalar.

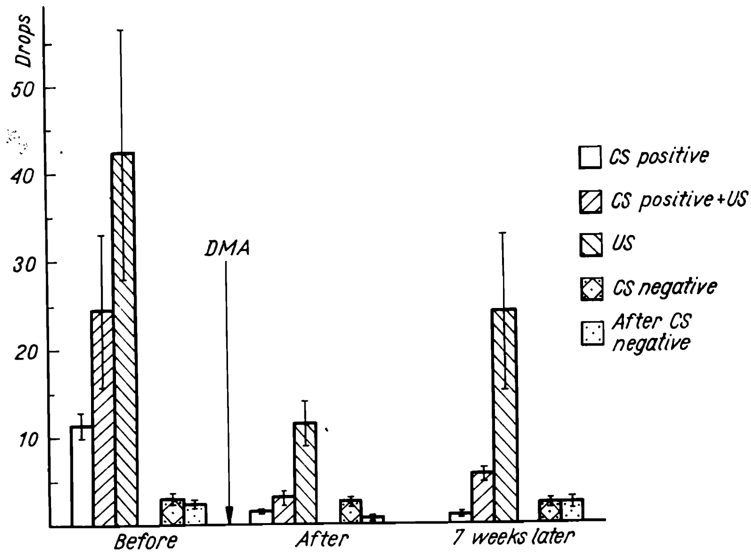

Fig. 3. Salivary reactions of the dog Melange in 10 successive experimental sessions. Columns represent mean value of reactions of 10 dogs during 10 days before, 10 days and 7 weeks after the operation.

The satistical analysis (Walsh test) indicates that the decrease of the conditioned and unconditioned salivation to CS^+ is significant for the group at the 0.05 level and that it is significant at 0.025 level for salivation during the differentiated CS^-.

Histology. Histological verification shows that in all the dogs the dorsomedial amygdala was destroyed completely or partially (Fig. 4). There were, however, differences in the localization and dimensions of the lesions among individual dogs. Melange had a bilateral lesion in the medial nucleus, anterior amygdala and substantia innominata and a unilateral lesion in the left basal magnocellular nucleus and right basal parvocellular nucleus. Mlano had a bilateral lesion in the medial, central and basal magnocellular nuclei and unilateral damage in the right basal parvocellular nucleus. Piaff had a unilateral lesion in the right central nucleus and left anterior amygdalar area as well as in the magno-and par-

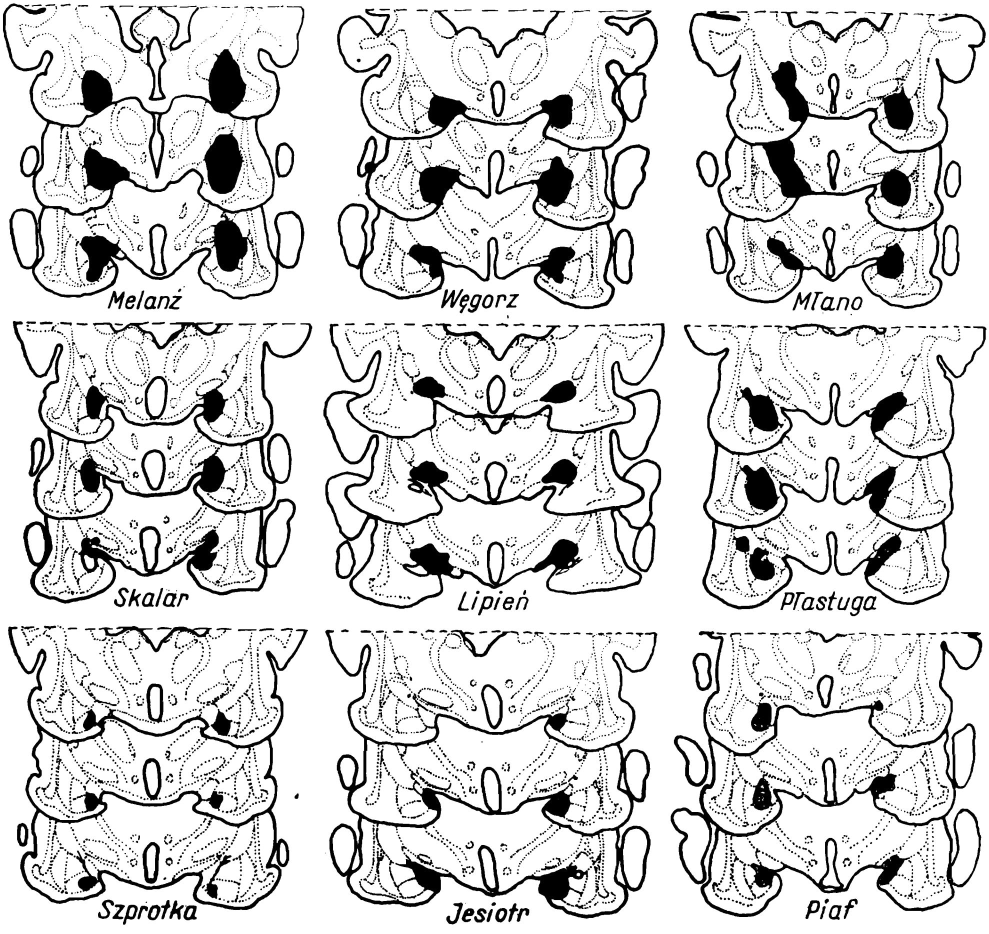

Fig. 4. Frontal sections of brains of operated dogs with dorsomedial amygdalar lesions.

vocellular basal nucleus. Lipień had a bilateral lesion in the medial nucleus and anterior amygdala. Skalar had a bilateral lesion in the medial nucleus and anterior amygdala. Płastuga had a bilateral lesion in nucleus centralis, nucleus medialis and unilaterally in the anterior area of the amygdala. Węgorz had a bilateral lesion in nucleus medialis, nucleus centralis and part of nucleus corticalis. Szprotka had a small bilateral lesion in nucleus corticalis, nucleus basalis parvocellularis in its ventral part and unilaterally in nucleus medialis. Jesiotr had a small bilateral lesions in central, medial and cortical nuclei and a big unilateral lesion in the thalamus.

DISCUSSION

The results of our experiment show that after dorsomedial amygdala lesions, salivary reactions to positive alimentary stimuli are decreased. This decrease seems not to parallel exactly the decrease in food intake because it appeared in dogs with pronounced aphagia and in dogs with only a small decrease of food intake. This might be explained by data obtained by Hainsworth and Epstein (6) and Kissileff (8), who interpreted the decrease of conditioned and unconditioned reactions as an impairment of the basic salivary mechanism, or impairment of the uncontitioned salivary-act mechanism (20) independent of the hunger drive. Konorski (10) thought of the salivary reactions, both conditioned and unconditioned, as typically "consummatory", only slightly dependent on the hunger drive. The independence of the hunger drive from salivary reactions has been described by Sołtysik (17), who found that satiated dogs consumed food slower than hungry ones and only because of it salivated less during certain units of time. He suggested that their consummatory mechanisms were impaired. However, four of our non-aphagic dogs showed a decrease of salivation although the speed of their eating seemed not to be changed. Our results showed decrease in salivation of dogs with a normal level of food intake, which indicates that consummation of food and salivation are two different mechanisms. Thus Sołtysik's explanation is not sufficient.

It is difficult to determine which amygdalar nuclei are most responsible for the impairment of salivary reactions. In those dogs in which the medial and basal nuclei and anterior amygdala were damaged, the impairment was the most pronounced. It is impossible to ascertain the role of the central and cortical nucleus because a pronounced decrease of salivation was observed in dogs with lesions in this nucleus as well as in dogs in which the damage did not involve these nuclei.

It is very difficult to compare the hypothalamic and amygdalar role in salivary reactions because previous data were obtained only from strongly aphagic lateral hypothalamic-lesioned dogs. In any case, we may say that both of these structure, the amygdala as well as the hypothalamus, are engaged in drive and vegetative aspects of alimentary behavior. Similarities between the behavioral symptoms of aphagia evoked by destruction of appropriate parts of both of these structures may indicate that the alimentary system works as a whole and damage of one of its links may evoke a very general effect so that integration of all aspects of alimentary function takes place on many levels of this system (3, 4).

Another interesting problem is that the animals with fairly similar amygdalar lesions showed different behavioral symptoms. Destruction of the dorsomedial part of this structure, which is the crucial locus involved in evoking aphagia (1–4), may evoke also other behavioral effects, such as depression, negativism, decrease of social reactions.

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Jadwiga ŁAGOWSKA and Elżbieta FONBERG, Department of Neurophysiology, Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warsaw, Poland.