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Ibotenic Acid Lesion of the Lateral Hypothalamus Increases Preference and Aversion Thresholds for Saccharin and Alters the Morphine Modulation of Taste

K. TOUZANI AND L. VELLEY[1]

Laboratoire de Psychophysiologie, URA CNRS 339, Université de Bordeaux I
Avenue des Facultés, 33405 Talence, France

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TOUZANI, K. AND L. VELLEY. *Ibotenic acid lesion of the lateral hypothalamus increases preference and aversion thresholds for saccharin and alters the morphine modulation of taste.* PHARMACOL BIOCHEM BEHAV **36**(3) 585–591, 1990.—In a previous study we showed that bilateral ibotenic acid lesions of the lateral hypothalamus in rats induced an increase in gustatory preference thresholds for saccharin solutions and which were associated with body weight and daily water intake impairments. The first aim of the present study was an attempt to dissociate the body weight and water intake deficits from the increase in gustatory thresholds. For this purpose we compared the effect of simultaneous bilateral lesions of the lateral hypothalamus with the effect of successive lesions in which each unilateral destruction was separated by a 10-day interval. Rats injected with vehicle only (either simultaneously or successively) served as controls. The two types of lesion produced very similar deficits, namely permanent body weight and water intake decreases, as well as a shift to the right in gustatory preference-aversion functions for saccharin (two-bottle procedure). The second aim of the present study was to analyse the effect of morphine (2 mg/kg SC) on saccharin preference in both lesioned and control rats. It was observed that for moderate and high concentrations of the sweetener morphine increased preference for saccharin over water but this effect was similar in both groups of rats. However, with a low concentration of the sweetener (0.3 mM) morphine clearly induced an opposite effect in the two groups of rats: the significant preference for this concentration shown by the control rats after vehicle injection was converted to a neutral response, whereas the neutral response of the lesioned animals after vehicle injection was transformed by morphine to a significant preference for saccharin over water. These results are discussed and in particular suggest that endorphinergic neurons located in the lateral hypothalamus are implicated in the modulation of palatability.

Lateral hypothalamus Intrinsic neurons Ibotenic acid lesion Saccharin Morphine Body weight Water intake Palatability Rat

IN a previous study, we tested the role of intrinsic neurons located in the lateral hypothalamus (LH) on gustatory preference-aversion functions for increasing concentrations of saccharin solution in the rat (13). The simultaneous bilateral lesion of these neurons by ibotenic acid (IBO) resulted in a significant shift to the right of the preference-aversion curve, suggesting that the rewarding and aversive values of taste stimuli are modulated by intrinsic neurons. However, the increase in preference thresholds was also associated with other deficits, namely a permanent body weight impairment, a permanent deficit in daily water intake and an increased neophobia on the first presentation of the saccharin solutions. Thus, it was not possible to conclude if the gustatory deficit was due to a disturbance of palatability or to the general decrease in water intake. The first aim of the present study was an attempt to dissociate the body weight and water intake deficits from the increase in gustatory thresholds.

In a recent electrical self-stimulation study (12) we observed that when two successive unilateral lesions of the LH are separated by a 10-day interval, neither body weight nor water intake deficits resulted. This suggested that a 10-day interval was sufficient to attenuate the deficits observed after a simultaneous bilateral lesion. Thus, in the present study we compared the effects of a bilateral ibotenic acid lesion of the LH performed either in the usual one-stage operation or in a two-stage operation, in which the contralateral LH was lesioned 10 days after the first operation.

The second purpose of the present study was to analyse the role of LH neurons in the opioid-mediated modulation of reward mechanisms implicated in gustation. As already indicated the shift in the gustatory preference-aversion functions following LH ibotenic acid lesion suggests that these LH neurons modulate the

[1]Requests for reprints should be addressed to L. Velley.

rewarding quality of sweet solutions. Moreover, in the self-stimulation study cited above we demonstrated that self-stimulation in the parabrachial area, which is the second relay station of ascending gustatory signals [reviewed in (14)] was greatly depressed after IBO lesion of the LH. If we suppose, as others have done (25), that self-stimulation in the parabrachial area results from the artificial activation of reward processes associated with certain taste cues, our results suggest that the intrinsic neurons of the LH are implicated, at least in part, in such reward processes. In parallel with these behavioral data, there now exists sufficient evidence to indicate that endogenous opioid systems are involved both in rewarding electrical brain stimulation [reviewed in (27)] and in reward mechanisms associated with taste [reviewed in (7)]. Consequently, in order to test the hypothesis that some LH neurons are implicated in the opioid-mediated modulation of palatability we compared the effect of morphine on saccharin preference in both lesioned and sham-lesioned rats.

METHOD

Animals

Male rats of the Sprague-Dawley strain (IFFA-CREDO, Lyon) were individually housed in wire-mesh cages and maintained on a regular 12:12 hr light-dark cycle in a temperature-regulated (21–23°C) animal room.

Surgery

When the rats were 2 months old, the intrinsic cells of the LH were lesioned by local injection of ibotenic acid. Under pentobarbital anesthesia (Nembutal 40 mg/kg IP) one cannula (outer diameter 0.24 mm) was successively implanted in each LH under stereotaxic control. The coordinates were 6.4 mm anterior to the interaural line, ±1.7 mm lateral to the sagittal suture and 9 mm ventral from the skull surface. The incisor bar was level with the interaural line. The cannula was connected with a micropump which delivered in each LH 4 μg of IBO in 0.5 μl of vehicle (phosphate buffer). Each injection lasted 6.5 min and 10 more min elapsed before removal of the cannula. Four groups of animals were constituted. The first group (N = 13) included the rats whose bilateral LH lesion was performed during the same operation (one-stage lesion). In the second group (N = 14) each unilateral lesion was separated by a 10-day interval (two-stage lesion). Two corresponding sham-lesioned groups of rats were injected with the vehicle either successively (N = 9, 10-day interval) or simultaneously (N = 9) and served as controls. The one-stage lesion and the one-stage injection of the vehicle were performed at the same time as the second unilateral lesion and the second vehicle injection of the two-stage operated rats. All rats were weighed daily from the fifth day before the first operation to the day of sacrifice.

Saccharin-Water Choice

Twenty-two days after the operation, all rats were placed on a schedule of restricted water access (4,13). Over 5 days, rats were allowed only two periods for drinking, one beginning at 0830 hr lasting one hour and one beginning at 1830 hr lasting 2 hr. After habituation to the deprivation-schedule, rats were presented with two bottles equipped with stainless steel drinking spouts, one containing tap water and the other containing one of the 6 concentrations of saccharin: 0.1, 0.3, 2.5, 7.5, 25 and 50 mM. Fresh solutions of the sweetener (sodium saccharin) were mixed daily by dissolving the tablets in tap water. The test session took place each day at 0830 hr and lasted 1 hour. The bottles were weighed at the beginning and at the end of the test to the nearest 0.01 g. The different concentrations of saccharin were tested one per day in ascending order. The bottle containing saccharin solution was placed on a different side of the home-cage every day. During the 6 days of testing the deprivation schedule was maintained as indicated above. Thus, the choice test took place after 12 hr of water deprivation.

Influence of Morphine on Gustatory Preference-Aversion Functions

At the end of the saccharin-water choice test water was given ad lib for 3 days. Then, all rats were again placed on the usual water deprivation schedule for 3 consecutive days. Three subgroups of lesioned rats and 3 subgroups of sham-lesioned animals were randomly constituted. Each subgroup was submitted to the saccharin-water choice test with one saccharin concentration only 0.3, 7.5 or 50 mM, during 4 consecutive days. For the 2 last days, thirty min before the test each rat was subcutaneously injected with the vehicle of morphine (1 ml/kg). The next day the same protocol was used but 2 mg/kg of morphine were injected 30 min before the test (4). Eight days later a second experiment was performed in all lesioned and control rats, in order to test more precisely the effect of morphine on the preference threshold with the lowest concentration of the saccharin solution (0.3 mM).

Histological Controls

At the end of the experiment with morphine, all rats were allowed to drink water ad lib for 3 days and were weighed daily. Then, they were killed by an overdose of Nembutal. The brains were frontally sectioned in a freezing microtome at 40 μm and stained with thionine. The extents of the lesions were reconstructed on the appropriate planes of the atlas of Paxinos and Watson (23).

RESULTS

Histological Analysis

The 27 brains of lesioned rats were analysed. A complete loss of neurons associated with glial proliferation was observed in the area of the lesion (Fig. 1B,C). For the two-stage lesioned rats the anteroposterior extent of the lesions varied between 1040 and 2160 μm (mean 1570 μm) and for the one-stage lesioned animals the extent varied between 1040 and 2080 μm (mean 1481 μm). In most cases the lesion began between frontal planes 6.70 and 6.88 of the Paxinos and Watson atlas (23), namely at the level of the caudal end of the paraventricular nucleus. The posterior limit of the lesions was observed between planes 4.84 and 5.20 at the frontal level of the premammillary nuclei. In the mediodorsal plane (Fig. 1A,B) the region extended from the perifornical region to the internal capsule. The dorsomedial nucleus was marginally damaged in 4 cases. In the dorsoventral plane the lesioned area extended from the bottom of the brain to the zona incerta. The zona incerta was damaged in most cases. In the thalamus a small lesion around the cannula track was observed in 13 brains, but no significant lesion was observed in the thalamic gustatory nucleus located in the posterior and ventral part of the thalamus.

Comparison of the Effects of the One-Stage vs. Two-Stage Lesions on Body Weight and Daily Water Intake

Two days after the simultaneous lesion the rats showed a weight loss amounting to 9.7% of the preoperative body weight. The first lesion in the case of the two-stage procedure produced a

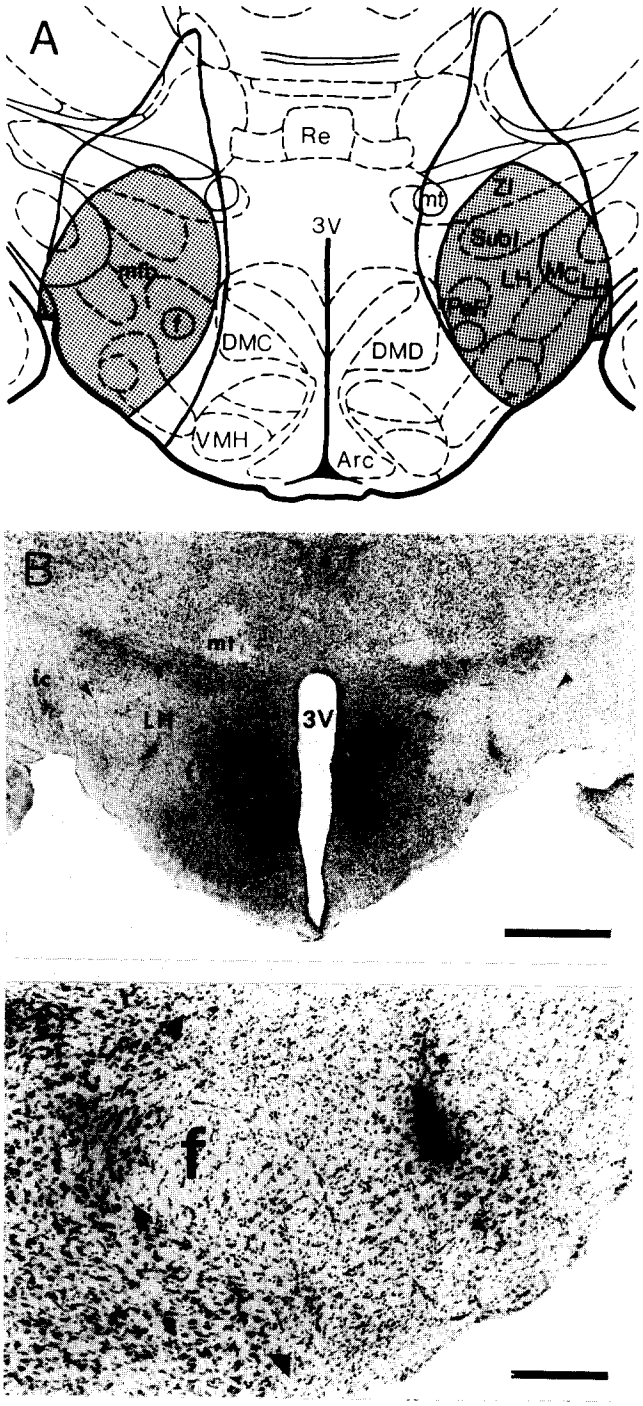

FIG. 1. (A) Schematic representation of the maximum extent of the lesions redrawn from the frontal sections on the plate 5.70 (interaural) of the Paxinos and Watson atlas (23). Dotted area represents the maximum extent of the lesions observed for 20 out of the total of 27 brains. For the 7 other cases, the larger lesioned areas are limited by the continuous line. Abbreviations: Arc: arcuate hypoth. nu.; DMC: dorsomedial hypoth. nu compact; DMD: dorsomedial hypoth. nu. diffuse; DMH: dorsomedial hypoth. nu.; f: fornix; ic: internal capsule; LH: lateral hypoth. area; MCLH: magnocellular nu. lat. hypoth.; mfb: medial forebrain bundle; mt: mammillothalamic tract; PeF: perifornical nu.; Re: reuniens thal. nu.; Sub I: subincertal nu.; VMH: ventro-medial hypoth. nu.; ZI: zona incerta; 3V: third ventricle. (B) Low-powered photomicrograph of the hypothalamic region showing an example of a bilateral lesion. The cannula tracks are visible. (C) Enlarged view centered on the fornix (f) to show the limit of the lesion. Scale bars: B: 1 mm; C: 0.2 mm.

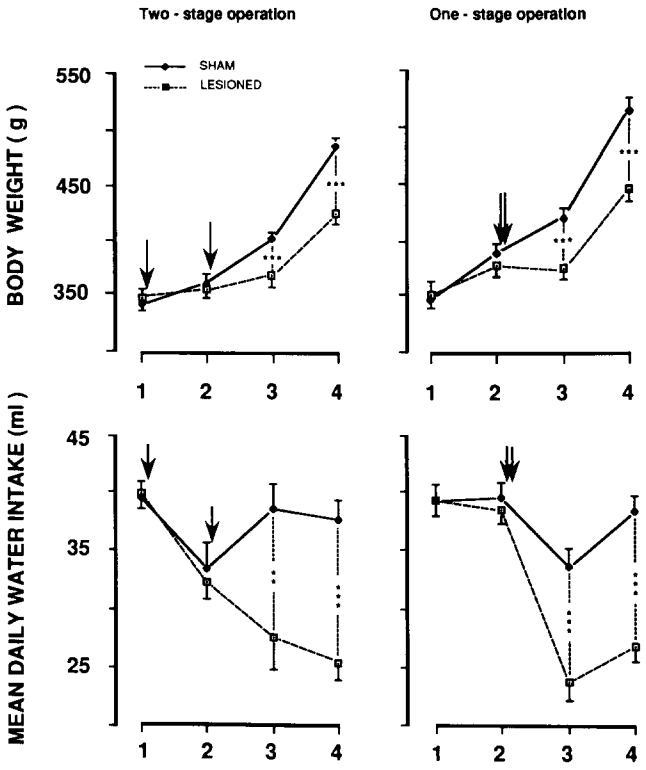

FIG. 2. Mean body weight (top) and mean daily water intake (bottom) after the two-stage operation (left) and after the one-stage operation (right). Four representative values are shown on the abscissa: 1: before the first unilateral operation (first single arrow); 2: before the second unilateral operation (second single arrow) and before the simultaneous operation (double arrow); 3: before the first restricted water access; 4: before the sacrifice of the rats. For this and the following figures the statistical significance is indicated as follows. $*p<0.05$; $**p<0.01$; $***p<0.001$.

weight loss which was 6.2% of the preoperative body weight. Ten days later the body weight of these rats was the same as their preoperative weight. Two days after the second lesion these rats showed a weight loss which was 7.4% of the preoperative body weight.

The top part of Fig. 2 shows the mean body weight increase of the 2 lesioned groups and of the corresponding 2 sham-lesioned groups. For clarity 4 representative values only are indicated: 1) mean body weight before the first unilateral lesion (first single arrow); 2) mean body weight before the second unilateral lesion (second single arrow) and before the simultaneous bilateral operation (double arrow); 3) mean body weight before the first restricted water access; 4) mean body weight before the sacrifice of the rats. A slight but significant difference appeared between the two sham-lesioned groups of rats, $F(1,16)=4.96$, $p<0.05$, whereas the body weight increase of the 2 lesioned groups was the same, $F(1,25)=2.8$, n.s. This last result indicates that the deficit produced by the lesion does not depend on the type of operation performed.

The bottom of Fig. 2 shows the effect of the lesions and of the sham operations on the mean daily water intake measured during the same period as for the body weight. Two main effects were observed. The operation per se, and not only the lesion, produced a significant decrease in daily water intake. This deficit was observed in the two groups of sham-lesioned rats and in the case of the two-stage operation the first unilateral operation was

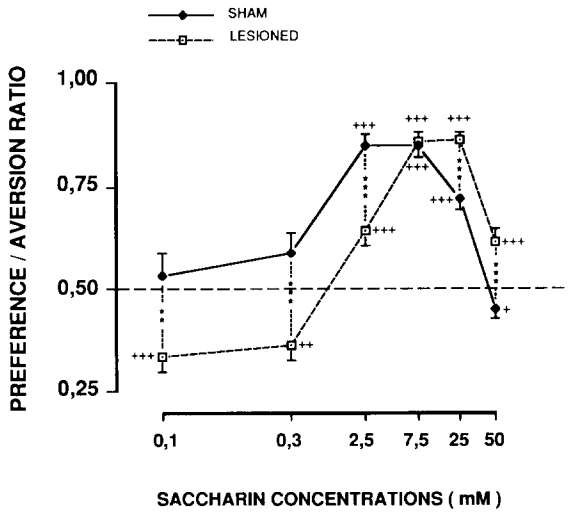

FIG. 3. Preference-aversion ratios for saccharin solutions of the two groups of rats. Two different statistical values are indicated. Asterisks show the significance between the preference-aversion ratio of the two groups for a given concentration value. Crosses show the significance between the preference-aversion ratio and the 0.5 value (no preference).

sufficient to induce this deficit. However, whereas in the two sham-lesioned groups normal water intake recovered, in the lesioned animals a significant water intake deficit was observed and the water consumption remained clearly inferior to that of sham-lesioned rats throughout the duration of their survival.

Saccharin-Water Choice

Given that the body weight and daily water intake deficits were the same after successive and simultaneous LH lesions, the 2 groups of lesioned rats were pooled as well as the 2 groups of the sham-lesioned animals.

Figure 3 allows a comparison of the effect of the lesion with that of the sham lesion on preference-aversion ratios, i.e., saccharin solution taken/total fluid taken during 1 hr. A 2×6 (group $\times$ concentration of saccharin) ANOVA was conducted to test the interaction between the lesion and the concentration of the sweetener. This analysis showed a significant group effect, $F(1,43) = 4.87$, $p<0.05$. The concentration effect was significant, $F(1,215) = 56.4$, $p<0.001$, as well as the interaction term, $F(1,215) = 14.28$, $p<0.001$, showing that the preference-aversion ratios were modified by the lesion. The highest preference score of the sham-lesioned rats was observed with a saccharin concentration of 2.5 mM, while the highest concentration (50 mM) was aversive. For the lesioned rats the two lowest concentrations were significantly avoided suggesting neophobia to the new solutions (cf. infra). All other concentrations from the 2.5 mM solution onward were significantly preferred to water. The highest preference score of the lesioned rats was observed with the 25 mM concentration (86.3) although a similar value was obtained with the 7.5 mM concentration (86.1).

Effects of Morphine on the Preference-Aversion Ratio to Saccharin

The results of this experiment are summarized in Fig. 4.

It can be observed that the aversive reaction of the lesioned rats on the first presentation of the 0.3 mM solution of saccharin (Fig. 3) disappeared in the present experiment after vehicle injection since the ratio was not different from the 0.5 value. For the control rats after vehicle injection the 0.3 mM solution was significantly preferred to water.

In the lesioned as well as in the sham-lesioned rats, morphine significantly increased the preference for the most concentrated solution (50 mM). The aversion for this concentration of saccharin of the control animals observed before morphine treatment was converted by the drug to a significant preference for the sweetener and the preference ratio of the lesioned rats was also significantly increased. For the most preferred solution (7.5 mM) the preference increase was small and nonsignificant in the 2 groups of rats.

The most significant result concerns the effect of morphine on

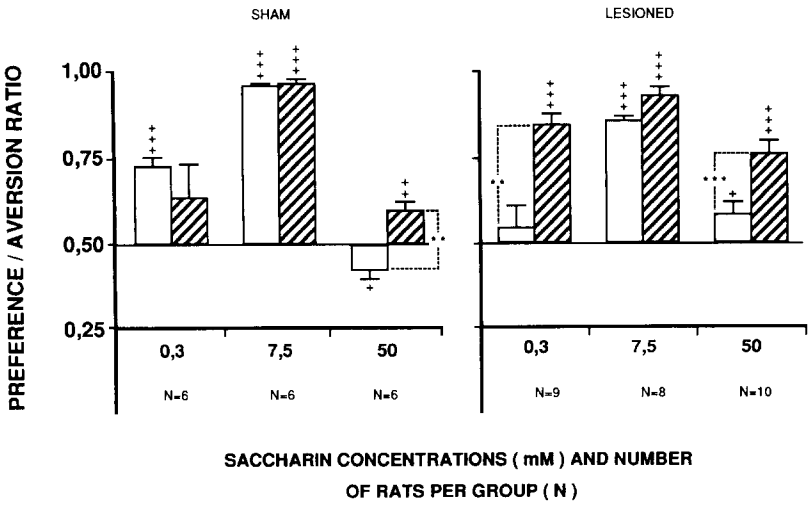

FIG. 4. Effects of the injection of morphine (2 mg/kg) on the preference-aversion ratios of 3 groups of lesioned and 3 groups of control rats. For each lesioned group and for the corresponding sham-lesioned group, one concentration only of saccharin was tested. Open bars: mean preference-aversion ratios measured during the two daily tests after vehicle injection. Hatched bars: mean preference-aversion ratios after morphine injection. Statistical values as in the Fig. 3.

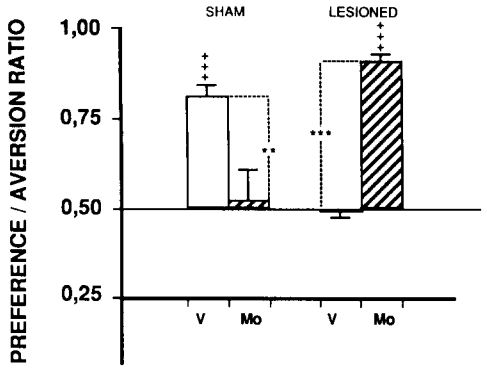

FIG. 5. Effects of the injection of vehicle (V) and morphine (Mo) on the preference-aversion ratios of sham-lesioned and lesioned rats for the 0.3 mM solution of saccharin. Statistical values as in the Fig. 3.

the preference-aversion ratio with the lowest concentration of the sweetener (0.3 mM). The significant preference of the control rats for this solution was converted to a neutral response after morphine, whereas in the lesioned animals the 0.3 mM solution which was neutral after vehicle injection, was significantly preferred following morphine injection. In order to verify this differential effect, all rats (lesioned and sham-lesioned) were allowed water ad lib for 8 days. They were then placed in the usual water deprivation schedule during 3 days and they were submitted to the saccharin water-choice test with the 0.3 mM solution of the sweetener, during 4 days. Thirty min before the two last daily tests, all rats received a SC injection of the vehicle. The next day they were injected with 2 mg/kg of morphine. The results of this experiment are shown in Fig. 5.

The data of the preceding experiment were clearly confirmed. The preference ratio of the control rats was converted into a neutral response, whereas the neutral response of the lesioned animals was transformed into a significant preference for saccharin.

DISCUSSION

As indicated in the introduction, the bilateral ibotenic acid lesion of the LH produces several deficits and the primary aim of the present experiments was to better understand the relationships between these different impairments. In disagreement with our hypothesis, the successive lesion of the LH did not alleviate the deficits observed after the simultaneous lesion. Both the body weight and the daily water intake disturbances were the same, suggesting that no recovery of function took place during the 10 days separating the 2 unilateral lesions. Reports concerning the effects of successive lesion of the LH have yielded conflicting results. Fass *et al.* (11) reported that removal of contralateral LH 30 days after the first unilateral lesion spares body weight deficits and the severe aphagia-adipsia which are associated with the simultaneous bilateral lesion. In contrast, Gold (15) and Almli and Golden (1) reported severe and persistent adipsia-aphagia and body weight deficits following two-stage bilateral lesion of the LH. The possibility that the lack of recovery observed in the present study was due to a too short interval between the two unilateral lesions seems to be unlikely since in the Almli study no recovery was observed despite the fact that 140 days separated the 2 unilateral lesions. Taken together, these data suggest that, at least in the case of the LH, the two-stage lesion procedure does not significantly suppress or attenuate the deficits observed after the usual one-stage lesion procedure.

However, these observations are not easy to reconcile with our previous data obtained in a self-stimulation study, which showed that no significant weight loss was observed after the second contralateral lesion (12). Two days after this lesion the body weight loss was 1.8% only and 4 days later a significant weight gain was observed (+5.6%). The differences between these previous results and the present data cannot be explained by differences in the location or in the extent of the lesions since in the two experiments the lesions were located in the same LH region and the extent of the damage was the same. The only difference between these two experiments is that in our previous study the effect of successive LH lesion was tested in a self-stimulation paradigm. The rats were intensively trained in self-stimulation before as well as after the first unilateral lesion. Thus, an intriguing possibility would be that this intense self-stimulation training may have reduced the regulatory deficits observed in the present study.

The present results concerning the preference-aversion functions for increasing concentrations of saccharin solutions are in agreement with our previous data (13), namely that the preference-aversion ratios of the LH-lesioned rats are displaced towards higher concentrations of the sweetener. Moreover, the data confirm that the lesion produced increased neophobia on first presentation of saccharin solution: the lesioned rats significantly avoided the two first presentations of saccharin, i.e., the 0.1 and 0.3 mM solutions (Fig. 3). However, when the 0.3 mM solutions were presented for the second and third times (Figs. 4 and 5), the response of the lesioned rats was neutral, whereas the control rats showed preference for saccharin over water.

The second purpose of the study was to investigate if some of the LH neurons destroyed by the IBO injection may be implicated in the opioid modulation of sweet-tasting solutions. A number of studies suggest a connection between endogenous opioid peptide mechanisms and sweetness palatability. It was demonstrated that highly palatable foods increase release of β-endorphin and decrease in vivo binding of ^{3}H-etorphine in the hypothalamus (9). By far, the most numerous studies have employed opiate antagonists and have shown that naloxone or naltrexone markedly attenuated consumption of preferred solutions and palatable nutrients [(5, 19, 21, 32) review in (7)]. The number of studies employing opiate agonists is much limited and the results are conflicting. Cooper [quoted in (6)] did not observe increased saccharin intake after morphine injection, whereas other authors found substantial increases of intake of the sweetener following morphine (4,8). Our results are partly in agreement with these latter observations. Morphine increased the preference for the most concentrated solution of saccharin (50 mM) and also produced a small nonsignificant increase of saccharin intake with the 7.5 mM solution. However, with these two solutions, the direction and the magnitude of the responses of the LH-lesioned rats were the same as those of the sham-lesioned rats despite the fact that the liquid intake of the lesioned animals was significantly inferior to the intake of control rats.

However a significant and differential effect of morphine was observed when the concentration of saccharin solution was near the preference threshold (0.3 mM). With this solution morphine suppressed the preference of the sham-lesioned rats for saccharin, whereas the neutral response of the lesioned rats was converted to a significant preference for the sweetener. The effect of the opiate agonist was clearly to produce a significant and opposite change in saccharin intake, although the near total suppression of water intake observed in the lesioned rats injected with morphine may result from the general impairment of liquid intake of these animals.

The results observed in the control rats indicate that the effect of morphine on saccharin intake depends on the concentration of the sweetener solution: the morphine-induced suppression of the preference for the 0.3 mM solution is probably due to the fact that this concentration is around the preference threshold for normal

rats. This somewhat paradoxical result showing that morphine behaved like an antagonist when saccharin concentration was at the preference threshold is difficult to explain at present. Recently, Lynch (21) showed a positive correlation between the concentration of the saccharin solutions and the dose of naloxone necessary to suppress preference for the sweetener. Although in this experiment the lowest concentration of saccharin used was well above the preference threshold, the result suggests that the activation level of opioid systems increases as a function of the concentration of saccharin. Thus, it is likely that at the concentation value used in the present experiment the activation effect of the opioid system was very low, and it can be postulated that the paradoxical effect of morphine may be due either to the presence of two different opioid systems operating antagonistically or to an interaction with presynaptic opiate receptors at low levels of neuronal activation. Although this latter possibility is highly hypothetical it is worth noting that low doses of naloxone have a paradoxical antinociceptive effect in some experimental models of pain and it was proposed that this effect would result from the stimulation of putative presynaptic opiate receptors (17,29). Whatever the value of this hypothesis, it will be necessary to extend our observations by employing opiate agonists and antagonists on the preference responses for solutions of saccharin of different concentrations both above and below the threshold value.

With regard to the lesioned rats it appears that the lesion suppressed preference for the 0.3 mM solution of saccharin and that after morphine this preference reappeared. This effect confirms that a fully functioning opioid system is essential to maintain the preference for saccharin when external incentives are weak. Furthermore, they suggest that some intrinsic neurons of the LH are implicated in this process. Immunocytochemical data have demonstrated that a significant number of enkephalin and dynorphin perikarya are located in the LH (10, 18, 24, 28). It is likely that among these endorphinergic neurons, those located in the middle and posterior parts of the LH were destroyed by the neurotoxin and consequently that some endorphinergic inputs to neurons involved in gustation disappeared following the lesion. A significant part of the LH neurons are known to project to the first and the second relay stations of the gustatory ascending signals, namely the nucleus of the solitary tract and the parabrachial area (3, 16, 20, 26, 30, 31). Moreover, electrophysiological data have shown that most gustatory neurons of the nucleus of the solitary tract are polysynaptically activated by LH stimulation (2,22). Recent retrograde tracing immunofluorescence technique demonstrated that LH cells immunoreactive to dynorphin project to these two taste areas (33). Taken together, these data suggest the possibility that some endorphinergic neurons in the LH modulate the activity of gustatory neurons in the two relay stations of the brainstem.

However, given the widespread projections of the LH neurons, the possibility that the modulation of the gustatory neurons take place in other brain areas outside the brainstem cannot be excluded. In order to overcome this difficulty we intend to analyse in lesioned and control rats the effects of local injections of opiate agonists and antagonists in gustatory brain areas known to receive significant projections from the LH neurons.

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