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Research report

The role of the external lateral parabrachial subnucleus in flavor preferences induced by predigested food administered intragastrically

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Abstract

A study was undertaken of the role of the external lateral parabrachial subnucleus (PBNLe) in flavor preferences induced by the intragastric administration of predigested/cephalic food. These preferences were developed using two different learning procedures, concurrent and sequential. In the concurrent procedure, two different-flavored stimuli were presented at the same time: one stimulus was paired with the simultaneous intragastric administration of partially digested food and the other with physiological saline. In the sequential learning procedure, the two stimuli were presented at alternate sessions. The results showed that PBNLe lesions blocked acquisition of concurrent learning but had no effect on the sequential procedure. In the latter case, both lesioned and control animals showed a strong preference for the gustatory stimulus paired with partially digested food. These results are interpreted in terms of a dual neurobiological system involved in the rewarding effects of visceral signals.

Theme: Neural basis of behavior

Topic: Ingestive behaviors

Keywords: External lateral parabrachial nucleus; Appetitive learning; Food intake; Rat

1. Introduction

The rewarding or aversive qualities of food, together with other more immediate factors such as taste properties [17], frequently depend on the animal's prior experience of the food, i.e., on an associative learning between the food and the consequences of its ingestion [6,17,50,51]. Thus, if consumption of a substance is followed by distress, this product is avoided at subsequent presentations [2–4,39,41,58]. On the other hand, positive post-ingestional effects of taste stimuli, such as the reduction of an energy deficit or recovery from a vitamin deficiency, are key to establishing taste preferences [6,9,23,50,51].

Previous studies have demonstrated that the intragastric administration of some nutrients usually leads to the development of strong taste aversions [16,50,51]. This is not the case with predigested food from donor animals, and this fact has been interpreted in terms of the cephalic phase of digestion [42,49,72], which comprises a series of digestive neuroendocrine secretions that result from stimulation by the food of the cephalic sensory systems, especially the oral–pharyngeal cavity [7,27,35,45,47]. According to pioneer research by Pavlov and subsequent studies, cephalic phase responses prepare the digestive system for an optimum utilization of the ingested nutrients [7,27,42,45,47,61]. Hence, nutrients that are administered intragastrically, in the absence of the cephalic phase, arrive at the gastric cavity in abnormal physiological conditions [45].

Abbreviations: AP/NTS, area postrema/nucleus of the solitary tract region; FLI, Fos-like immunoreactivity; NTS, nucleus of the solitary tract; PBN, parabrachial nucleus; PBNL, lateral parabrachial nucleus; PBNLe, external lateral parabrachial subnucleus

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As several studies have demonstrated, two-choice preference tests constitute a sensitive and reliable measure for both the aversive [16,25] and rewarding properties [50,51,58] of an intragastric stimulus. Previous studies have used concurrent (short-term) [50,51,72] and sequential (long-term) learning procedures to establish taste preferences [58]. In concurrent learning, two flavor stimuli are offered at the same time for several minutes: the ingestion of one flavor is paired with the simultaneous intragastric administration of predigested food, whereas ingestion of the other is associated with an innocuous non-nutritive product, such as physiological saline [50,51,72]. In the sequential learning procedure, two different-flavored stimuli are presented on alternate days [14,56–58]; one stimulus is associated with the intragastric administration of a predigested nutrient and the other with physiological saline [50,51,72].

One major difference between concurrent and sequential learning is that in the former, and provided that the animals alternate between both gustatory stimuli, the animal must detect and process the visceral stimulus rapidly for it to be associated with one of the two flavor stimuli. This feature appears in turn to determine which neural structures are involved in one or other learning modality. Recent studies carried out by our group suggested that the vagus nerve could constitute this rapid transmission pathway [72], as was also reported in studies on taste aversion learning [2–4]. On the other hand, this neural pathway does not appear to play a major role in sequential flavor learning, whether of a rewarding [72] or aversive nature [2–4,39]. Sequential learning appears to depend upon the blood circulatory system and circumventricular structures, such as the area postrema [21,22,54].

Several anatomical and functional studies have demonstrated that vagal afferents from the digestive system terminate in parts of the nucleus of the solitary tract (NTS) [1,48,59,65,73,74]. In turn, these subnuclei, in addition to some area postrema [20,31] and splanchnic afferents [10,11,71], mostly project to the lateral division of the parabrachial complex (PBNL) and especially to the external lateral subnucleus (PBNLe) [20,31]. This anatomical organization is consistent with findings that showed the activity of this subnucleus to be modified both after electric stimulation of the vagal nerve and after visceral manipulations [28,32,36,56,64,66–70]. Likewise, PBNLe exhibits Fos-like immunoreactivity to both the intraduodenal administration of glucose [64] and the intragastric administration of noxious substances such as ethanol or lithium chloride [56,66,68] or nutrients such as lactose, sucrose, glucose, maltose or polycose [66,67]. On the other hand, the total number of c-FLI neurons expressed in PBNLe decreases after sucrose infusion and vagotomy [66].

Given these anatomical connections, it can be hypothesized that the PBNLe may play a role in the concurrent learning of flavor preferences. Indeed, it has recently been demonstrated that PBNLe lesions block taste aversion learning in concurrent paradigms when the visceral noxious stimulation is induced by substances such as hypertonic NaCl [41], whose processing through the vagus nerve seems to be well established [5,12,13,29]. In contrast, this subnucleus appears to play no role in sequential taste aversion learning [41].

The PBNLe, in common with most of the parabrachial complex, participates in many regulatory and learning processes, including those involved in food intake [8,33,34,38,52,53,63]. This subnucleus has been implicated, together with the vagal nerve, in feeding behavior elicited by agents such as 2,5-anhydro-D-mannitol (2,5-AM) or mercaptoacetate (MA) [8,33,34,37,52,53]. This feeding response can be blocked both by vagotomy or perivagal capsaicin treatments [8,37,52] and by electrolytic lesions of the PBNLe [8]. In addition, the peripheral administration of either agent (2,5-AM or MA) induces expression of Fos-like immunoreactivity in the PBNLe [8,33,34,52,53], whereas this activity and its effect on feeding is virtually abolished by subdiaphragmatic vagotomy [8,52,53].

Considered together, the above data suggest that some of the visceral information from the digestive system may be transmitted through the vagal nerve to the external subnucleus of the lateral parabrachial nucleus after its anatomical connection in the NTS. This neural pathway, defined either anatomically or behaviorally, appears important in processes linked to eating behavior and perhaps also in processes related to the development of flavor preferences induced through the intragastric administration of rewarding predigested nutrients [50,51]. Thus, the objective of the present experiment was to examine the effect of lesions in the PBNLe in preference gustatory learning, induced by the intragastric administration of predigested foods as visceral stimulus. We hypothesized that PBNLe lesions might interrupt the acquisition of gustatory preferences induced by rewarding foods in tasks of concurrent learning, as occurs after vagotomy in flavor preference studies [72] and after lesions of the vagal nerve or PBNLe in taste aversion learning [2–4,40].

2. Experiment 1

2.1. Materials and methods

2.1.1. Subjects

Nineteen naïve male Wistar rats (260–300 g each at the beginning of the experiment) from the breeding colony at the University of Granada were used in this study. They were randomly assigned to one of two groups: 10 were included in the lesioned group and nine in the control group. Ten neurologically intact donor rats from previous experiments were additionally used to provide the predigested nutrients. Subjects were individually housed in

methacrylate cages (30×15×30 cm), which also served as training chambers during the experiment. The sides of the cages were black and opaque; the front and back sides were transparent. The front side had two 1.6-cm holes at the same distance from the center and edges and at the same height above the floor of the cage. Through those orifices, the animal had access to spouts attached to cylindrical graduated burettes, through which the flavors, liquid diet, and water were administered.

The room temperature was maintained between 21 and 23 °C and the light–dark periods were 12 h each. All handling was done during the light phase. The animals were allowed a 2–3-day adaptation period, during which time they remained in their cages and had free access to food (Panlab, Barcelona) and water. All behavioral procedures and surgical techniques were conducted in accordance with the animal care guidelines established by the Spanish Royal Law, 223/1988.

2.1.2. Surgical procedure

2.1.2.1. Electrolytic lesions of the external lateral parabrachial subnucleus (PBNLe). Surgery was carried out under general anesthesia with sodium pentothal (50 mg/kg, Lab. Abbot, Spain). Once the animals were anesthetized, they were placed in a stereotaxic device (Stoelting Stereotaxic 51.600). Cathodic electric current (0.3 mA) was bilaterally applied for 10 s, using a DCML-5 lesionmaker (Grass Instruments, Quincy, MA, USA) that supplied current through a stainless steel monopolar electrode approximately 200 μm in diameter and insulated throughout its length, except for the last 0.5 mm. The anatomical coordinates for the PBNLe were obtained (interaural references) from the Paxinos and Watson stereotaxic atlas [46]: anterior/posterior (AP)=−0.16 mm; lateral (L)=±2.5 mm; and ventral (V)=+3.0 mm. All of the steps described above were followed for the sham lesion control group except that a vertical coordinate of +4.0 mm was used and no current was applied.

2.1.2.2. Intragastric catheters. Two intragastric catheters were implanted using a modified version of the procedure developed by Deutsch and Koopmans [15]. In brief, two silastic tubes (Silastic, Silastic-Medical Grade Tubing, Dow Corning, MI, USA) were implanted into the cardiac portion of the stomach and were routed through the abdominal muscle wall and placed under the skin, one on each side of the animal, at the back of the neck. Stitching was performed as needed to help close the wounds, and the rats received an intramuscular 0.1-cc dose of penicillin (Penilevel Retard. Lab., Level, Barcelona) as a prophylaxis against infection. This same procedure was used for the non-lesioned (control) and donor rats.

2.1.3. Behavioral procedure

A period of 13–14 days was allowed for postoperative recovery with free access to food and water. After this time, the lesioned and control subjects underwent a 4-day pre-training period. During each pre-training session, all animals were water-deprived and were allowed to daily drink tap water from graduated burettes offered simultaneously (to avoid positional preferences) through the frontal orifices in the cages. Water was provided to the subjects for 10 min on the first 2 days and for 7 min on the last 2 days. Thirty minutes after removal of the water, the animals were provided with 15 g of food (Panlab). All food that had not been consumed was removed in the evening of the fourth day. The donor rats were placed in a different room and were trained for 5 days to ingest a liquid diet (Ideal Evaporated whole milk, 50% diluted; Nestlé, Barcelona); 100 ml of this liquid diet contained 5.75 g of carbohydrates, 3.93 g of fat, and 3.93 g of protein (total energy, 74.37 kcal). The diet was offered for several hours both in the morning and afternoon. During the first 2 days of the pre-training period, this diet was combined with approximately 7 g of solid food. During the three remaining days, the subjects were only provided with a liquid diet. Water was offered for 10 min during the evening of each day, although the animals did not generally consume it.

The experiment began after the 4-day pre-training period, when the lesioned and control animals had learned to drink alternating between the two burettes. The rats were given a choice in each trial of two gustatory stimuli offered at the same time (0.5% strawberry (S) and 0.5% coconut (C), McCormick, San Francisco, CA) for 7 min. Ingestion from one 0.1-cc-graduated burette was paired with the simultaneous intragastric injection of a partially digested liquid diet pumped out from the stomach of the donor rats (the food remained in the stomach of the donor rats at least 30 min before being extracted) [42]. The ingestion from the other burette was paired with physiological saline (PS, Apiroserum Lab., YBIS, Madrid) and simultaneously administered via the second catheter (see Table 1). The liquid diet and PS were intragastrically and simultaneously injected each time the rats drank from the associated burette, at a rate of 1 ml/1 ml of ingested flavor stimulus. In order to control any flavor preferences, the

Table 1
Diagram showing the balanced experimental conditions in the concurrent behavioral procedure (Experiment 1)

	Days 1–4
Half the animals (50% lesioned +50% controls)	S(l)+PD(i.g.) C(r)+PS(i.g.)
Half the animals (50% lesioned +50% controls)	S(l)+PS(i.g.) C(r)+PD(i.g.)

S(l), strawberry on the left; C(r), coconut on the right; PD(i.g.), predigested diet intragastrically administered; PS(i.g.), physiological saline intragastrically administered.

paired liquid diet was balanced so that half of the animals received the liquid diet when they drank S and received PS when they drank C. The other half received the liquid diet paired with C, and the PS paired with S. A graphic illustration of this procedure was previously published [40]. After 60 min, the experimental and control subjects were provided 10 g of food; later that evening, any remaining food was removed. The experimental procedure was repeated over the course of four trials.

2.1.4. Histology

After concluding the behavioral procedures, the animals in the lesioned group were deeply anesthetized with a sodium pentothal overdose and were intracardially perfused with isotonic saline followed by 10% formaldehyde (Formaldehyde, Probus, Badalona). The brains were then extracted and stored in formaldehyde for at least 1 week. They were then sectioned in the coronal plane at 40 μm on a vibratome. Slides were set and stained with cresyl violet and examined through a microscope to determine the location and extent of the lesions.

2.2. Results

2.2.1. Behavioral results

Two animals from the lesioned group and one from the control group were excluded during the experimental procedure because one of the catheters became detached. The total amounts consumed by the lesioned group ($n=8$) and the control group ($n=8$) in four sessions were analyzed using a three-way ANOVA (group×day×substance). The results showed that the effects of the nutrient ($F(1,14)=5.97$; $P<0.02$) and day ($F(3,42)=4.88$; $P<0.005$) were significant. The ANOVA also revealed a non-significant group effect ($F(1,14)=2.41$; $P<0.14$), i.e., that the total consumption was similar for all lesioned and control animals. This suggests that was no impairment in motor and/or motivational functions among the lesioned animals. The stimulus ingestion by each group was analyzed using a two-way ANOVA (days×substance). The result of this analysis revealed that the lesioned animals were unable to learn the task (Fig. 1A). In fact, only the effect of the day was significant ($F(3,21)=3.39$; $P<0.03$). The effects of the substance ($F(1,7)=0.57$; $P<0.47$) or interaction of days and substance ($F(3,21)=0.49$; $P<0.69$) were not significant. In contrast, the sham-lesioned control group learned the discriminatory task correctly (Fig. 1B), because the animals consumed significantly more of the gustatory stimulus paired with the liquid diet ($F(1,7)=8.91$; $P<0.02$).

2.2.2. Histological results

The microscopic analysis of the coronal sections taken from the PBNLe-lesioned animals revealed that the electrolytic lesions were centered on the PBNLe in all cases (Fig. 2).

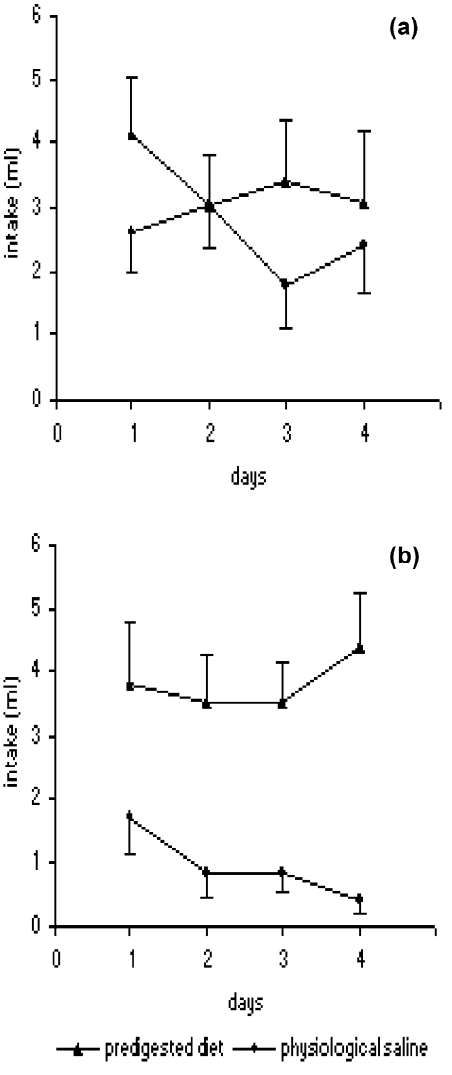

Fig. 1. Mean intake of gustatory stimuli paired with predigested liquid diet and physiological saline in PBNLe-lesioned (A) and control (B) rats of Experiment 1.

2.3. Discussion

The present data show that PBNLe lesions impair the acquisition of concurrent flavor learning induced by the intragastric administration of partially digested nutrients. Lesioned animals are unable to discriminate which of the offered gustatory stimuli is simultaneously associated with intragastrically administered nutrient (Fig. 1A). However, intact subjects correctly complete this task and develop a clear preference for the gustatory stimulus paired with injected foods (Fig. 1B). This result is consistent with previously reported data [50,51,72]. The failure of lesioned rats to acquire a flavor preference does not appear to be the

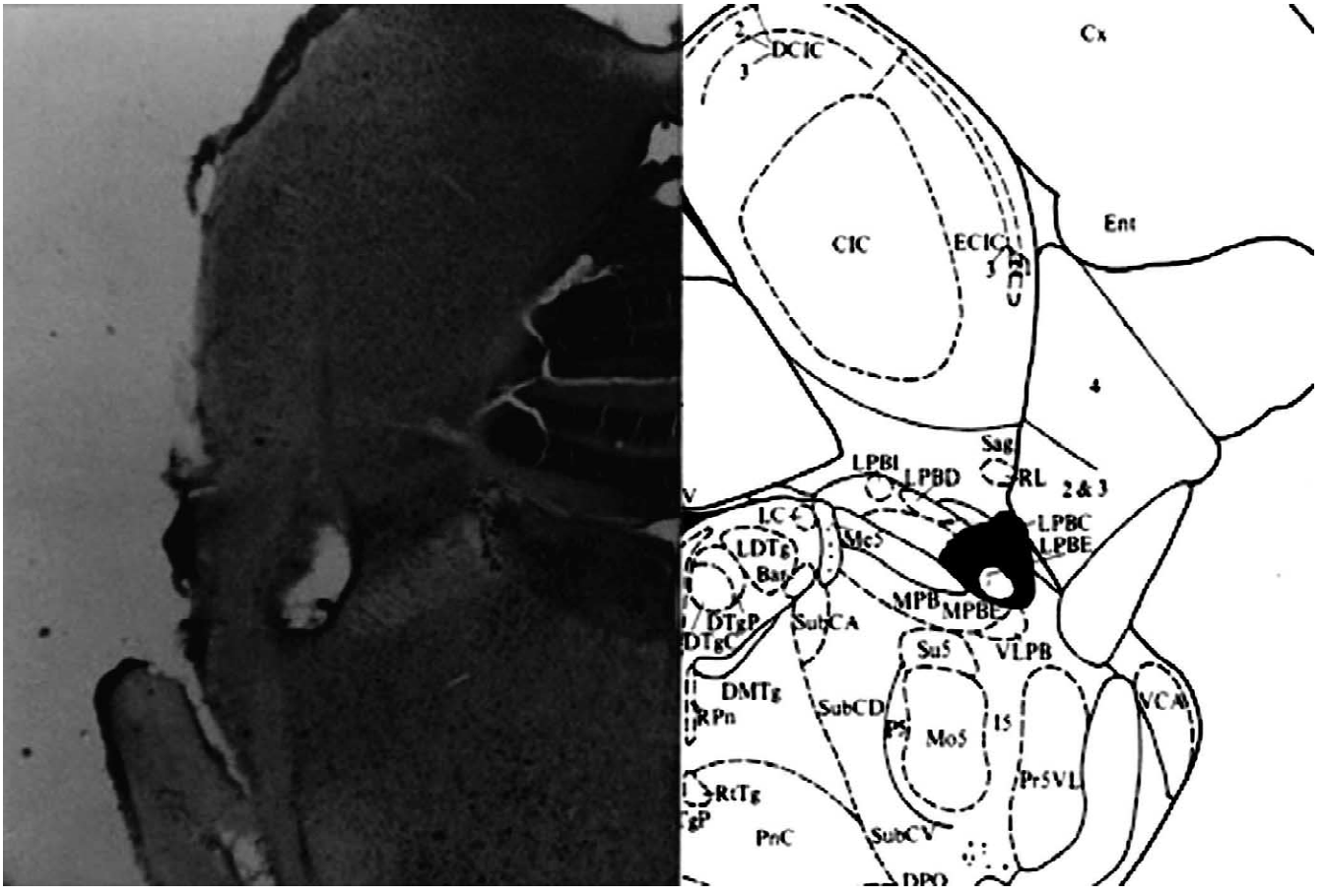

Fig. 2. Representative photomicrographs of coronal sections showing electrolytic lesions of the external lateral parabrachial nucleus (PBNLe), which were effective in blocking concurrent taste learning and ineffective in sequential taste learning. The schematic illustration was adapted from Paxinos and Watson's Atlas [46] and represent the largest (shown by black area) and the smallest PBNLe lesions (central white area).

result of a general behavioral impairment because their total intake was comparable to that of controls.

3. Experiment 2

Studies performed in the taste aversion learning field have demonstrated that this type of learning can be established through two different procedures: the concurrent procedure described in experiment 1 [2–4,16,40,41] and the sequential procedure [2–4,21,22,39–41,56,57,70]. The anatomical structures involved in each of these learning modalities seem to be different. For example, whereas the PBNLe appears to be a crucial nucleus in concurrent taste aversion learning, it is considered to have no role in sequential learning (the contrary to that observed when considering other nuclei such as the area postrema) [3,18,21,22,41,54].

Parallel to the research mentioned above, the present study was intended to verify whether the anatomical and functional dissociation established for sequential taste aversion learning is found when the visceral stimulation is generated through the intragastric administration of rewarding predigested nutrients, a procedure which establishes learned flavor preferences. This dissociation was observed in the latter context following the interruption of the vagal pathway [72]. Thus, the present experiment was designed to examine the role played by the PBNLe nucleus in sequential flavor preference learning induced by the intragastric administration of predigested nutrients.

3.1. Materials and methods

3.1.1. Subjects

Subjects were 19 male Wistar rats from the breeding colony of the University of Granada. These animals, weighing between 265 and 300 g at the beginning of the experiment, were randomly assigned to one of two groups, a PBNLe-lesioned group ($n=10$) and a sham-lesioned control group ($n=9$). Twelve donor rats, all neurologically intact from other studies, were also used and provided the

predigested nutrients. The animals were housed in the same conditions as described in experiment 1.

3.1.2. Surgical procedure

Identical to the procedure described in experiment 1.

3.1.3. Behavioral procedure

After surgery, all of the animals were allowed a recovery period of 8–11 days, with food and water available ad libitum. They then underwent a 4-day adaptation period during which they were water-deprived. During days 1–3, the animals had access to only one burette containing tap water, whose position was varied appropriately in order to avoid the development of positional preferences. On the last day, day 4, both burettes were simultaneously presented. On days 1 and 2, the water was offered for 10 min, while on days 3 and 4, the water was offered for only 7 min. Each day, 30 min after removal of the water, 15 g of solid food (Panlab) were administered and the experiment proceeded as described for experiment 1, except that on the day of the test, the donor animals were offered liquid food to satiation for several hours during the morning and afternoon. On the test day, no extractions were carried out, because the choice between the two gustatory stimuli was carried out without intragastric injections.

After 4 days of pre-training, the experimental learning test began. During this period, the animals passed through a four-session cycle and one final test. During the first and third sessions of this cycle, the animals were offered a graduated burette for 7 min, situated on the left side of the cage, which contained a gustatory stimulus (0.5% strawberry). Flavor intake was associated in half of the lesioned and control animals with a simultaneous intragastric injection of partially digested liquid diet, pumped out of the stomach of the donor rats. The other half of the animals received physiological saline. During sessions 2 and 4, which also lasted 7 min, those animals that had received predigested liquid food were presented with a different gustatory stimulus (0.5% coconut) in the right orifice, associated with the simultaneous intragastric administration of physiological saline. The animals that were previously administered physiological saline received a predigested liquid food (see Table 2). The administered volume of each product (liquid diet or physiological saline) was of 1 ml/1 ml of flavor stimulus ingested. After the four-session period (two learning trials), a choice test was performed in which both gustatory stimuli were simultaneously presented for 7 min (S and C), each in its respective position (Table 2). Each day at 60 min after the drinking period, the experimental and control subjects were provided with 10 g of food and the experiment proceeded as described for experiment 1. This two-trial cycle, in addition to the final test, was repeated a second time for a total of four learning trials.

3.2. Results

3.2.1. Behavioral results

Two animals from the lesioned group and one from the control group were excluded during the experimental procedure because the catheters became detached. Data from the choice tests were analyzed using a two-way ANOVA (group×substance). This ANOVA revealed that the effects of the group ($F(1,14)=2.59$; $P<0.12$), substance ($F(1,14)=0.03$; $P<0.85$), and group and substance interaction ($F(1,14)=0.007$; $P<0.93$) were not significant in the first choice test (after two learning trials). In contrast, the ANOVA of the second choice test showed a significant substance effect ($F(1,14)=27.13$, $P<0.0001$), indicating that both lesioned and control animals were able to acquire the learning task and showed a preference for the stimulus paired with partially digested liquid diet (Fig. 3). However, the group effect ($F(1,14)=1.94$; $P<0.18$) was not significant, showing that the total consumption was similar for lesioned and control animals. This suggests that there was no impairment of motor and/or motivational functions in PBNLe-lesioned animals. Furthermore, independent one-way ANOVAs for each group indicate a significant substance effect in both the lesioned ($F(1,7)=44,11$; $P<0.0002$) and non-lesioned control animals ($F(1,7)=6,98$; $P<0.03$).

3.2.2. Histological results

The histological analysis confirmed that the electrolytic lesions were centered on the PBNLe (Fig. 2) in all cases.

Table 2
Diagram showing the balanced experimental conditions in the sequential behavioral procedure (experiment 2)

	Trial 1		Trial 2		Choice test
	Session 1	Session 2	Session 3	Session 4	Session 5
Half the animals	S(l)+PD(i.g.)	C(r)+PS(i.g.)	S(l)+PD(i.g.)	C(r)+PS(i.g.)	S(l)−C(r)
Half the animals	S(l)+PS(i.g.)	C(r)+PD(i.g.)	S(l)+PS(i.g.)	C(r)+PD(i.g.)	S(l)−C(r)

S(l), strawberry on the left; C(r), coconut on the right; PD(i.g.), predigested diet intragastrically administered; PS(i.g.), physiological saline intragastrically administered.

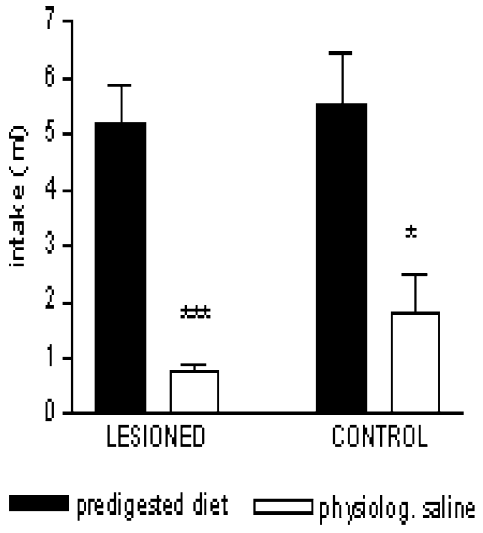

Fig. 3. Mean consumption of the gustatory stimuli paired with predigested diet and physiological saline during the second choice test in lesioned and control groups in Experiment 2. *$P<0.05$; ***$P<0.001$.

3.3. Discussion

The results obtained in this study demonstrate that PBNLe lesions do not interfere with the learning of sequential flavor preferences. As can be observed in Fig. 3, the lesioned and neurologically intact animals were equally effective in learning. As a result, after four trials of stimuli association (gustatory with its respective intragastric administrations), all groups showed a strong preference for the flavor associated to the predigested food.

4. General discussion

The studies presented in this paper reveal that PBNLe lesions impair concurrent flavor preference induced by rewarding predigested food administered intragastrically. These lesions have no effect on sequential learning, because lesioned animals learn the task as effectively as do the neurologically intact subjects.

These results are similar to those obtained after the interruption of the peripheral vagal pathways. Thus, it was demonstrated that the interruption of the vagal fibers blocks concurrent learning but not sequential learning when taste aversions are induced by noxious visceral stimuli [2–4] or when flavor preferences are induced by the intragastric administration of predigested foods [72]. Similarly, PBNLe lesions have been shown to disrupt taste aversion learning when a concurrent procedure is used and when the visceral substances administered (NaCl) are stimuli that can be rapidly processed by the vagus [5,12,13,29]. In contrast, lesions of this subnucleus do not impair learning if the acquisition process is of a sequential type [41]. These studies seem to suggest that concurrent taste learning, whose main requirement is the rapid processing of visceral stimuli, is mediated by a neural axis that may include the vagal sensory pathway and, among others, the PBNLe. Consequently, critical lesions of components of this neural system could interrupt concurrent learning without affecting sequential learning.

This neural system also seems to participate in other mechanisms related to feeding behavior. Thus, agents such as 2,5-AM or MA, which stimulate food intake [8,33,34,37,52,53], have been shown to require an intact vagus nerve, as well as an intact AP/NTS or PBNLe. Furthermore, the actions of these drugs are blocked after vagotomy or capsaicin vagal deafferentation [52,53] and after PBNLe electrolytic lesions [8]. Cholecystokinin (CCK) is another chemical substance related to food intake that appears to require the integrity of the above-mentioned structures. CCK is a peptide hormone that has been implicated in satiation [26]. Intraperitoneal administration of this hormone has been demonstrated to induce expression of FLI in the NTS as well as in the PBNLe [19,55], whereas vagus nerve lesions [60] and NTS lesions block CCK-induced effects [43]. These studies and the data derived from experiment 1 support the implication of the PBNLe in the processing of visceral information transmitted by the vagal pathway. However, this nucleus does not appear to be essential in sequential taste aversion learning, an acquisition process that may include blood-borne signals initially processed through the area postrema [14,21,22,44,54].

The impairment of the concurrent flavor preference shown by the PBNLe-lesioned animals could also be due to an interruption of the taste stimuli or a blockade of viscerogustatory associative mechanisms. One requirement that any anatomical structure must fulfill to be considered as a visceral-taste stimulus integration center is the convergence of both sensory modalities in the structure [24,25,57,67]. The PBNLe appears to meet this condition [30,31,62,67,70]. Although the existence of visceral information in this subnucleus has been known for some time [20,31,56,64,68–70], the presence of gustatory information in the PBNLe has been demonstrated only recently [30,62]. These neurons mainly respond to negative hedonic or aversive stimuli, as in the case of quinine or HCl [30,62,67,70]. They are located in the caudal regions of the PBNLe and specifically in its internal portion [30,67]. In contrast, neurons responding to visceral stimulation are located in the external area of the subnucleus and in the rostral part [66–70].

Although the lesions in our study are placed at the rostral level of the PBNL (see Fig. 2), we cannot rule out the possibility that our lesions could have affected the gustatory cells related to negative hedonic stimuli (which were not employed in this study). Yamamoto and associates believe that this zone is related to visceral information processing, because it is specifically activated by stimuli (positive hedonic or aversive) of this nature [66–70]. However, unlike the case of stimuli such as

quinine or HCl, which activate the PBNLe subnucleus [30,62,66–70], oral stimulation with substances such as sucrose, saccharin or polycose, all strongly preferred by subjects, induce FLI activity in other subnuclei of the PBN but hardly any FLI activity in the PBNLe [66,67,70], unless these stimuli are transformed into negative hedonic stimuli by exposure to an aversive conditioning [70]. In fact, in the present study, the neutral gustatory stimuli were converted after learning into clearly preferred stimuli that do not seem to involve the PBNLe. Furthermore, and in agreement with the previous discussion, experiment 2 of this study demonstrates that the lesioned animals continue to adequately process the taste–olfactory stimuli, because they can correctly perform the sequential learning task. Thus, the interruption in concurrent learning cannot be attributed to a blockade in the transmission of gustatory information. Similarly, and in agreement with previously mentioned studies [30,62,66–70], it is probable that the gustatory information used in our experiments did not reach the PBNLe, so that the necessary sensory convergence would not have been produced. Therefore, it is unlikely that the observed disruption in learning can be explained as a result of an associative deficit. If the external subnucleus of the parabrachial lateral participates in some type of visceral-taste association, it would be related to aversion learning processes [69,70] rather than to taste preference learning. However, even in aversion learning it has been recently shown that lesions of the PBNLe do not disrupt sequential taste aversion learning or, therefore, the processing of taste stimuli [41]. In any case, we believe that these controversial interpretations should not be considered as conclusive. On the contrary, they should be considered with maximum caution at the present time.

In summary, the studies presented here indicate that the PBNLe is a critical anatomical structure in the modality of rewarding gustatory learning in which a rapid processing of visceral stimuli is required (concurrent learning). In contrast, this subnucleus is not essential when the task can be carried out in a more delayed form (sequential learning). In the latter case, other structures must be involved.

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