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Lesions of the lateral parabrachial area block the aversive component and induced-flavor preference for the delayed intragastric administration of nutrients in rats: Effects on subsequent food and water intake

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Abstract
The aim of this study was to examine the function of the lateral parabrachial area (LPB) in relation to the intragastric administration of nutrients. The consumption of flavors associated with intragastric nutrient administration and the subsequent food and water intake were measured in rats with lesions in the LPB. The results showed that bilateral LPB lesions prevented development of aversions and induced flavor preference when there was a delay between the presentation of a flavor and the intragastric administration of nutrients. However, these lesions did not disrupt development of the aversive process when there was no delay between the presentations. Likewise, the LPB lesions increased subsequent food intake when there was a delay but not when there was no delay between the presentations. In contrast, the water intake was reduced in both situations. These results are interpreted in terms of a dual visceral system for processing the intragastric effects of foods.

Keywords: *Intragastric nutrients, food intake, water intake, flavor aversion, lateral parabrachial area*

Introduction

Food intake is a complex process and both central and peripheral factors are involved in its regulation. Thus, numerous studies have demonstrated that the gastrointestinal system participates in satiety processes (Chernigovskii 1962; Davis and Campbell 1973; González and Deutsch 1981; Phillips and Powley 1998; Phiffer and Berthoud 1998; Schwartz 2000; Cox et al. 2004; Ritter 2004). Various approaches have been adopted to study this participation. One of the most frequent procedures is the direct administration of nutrients into different segments of the digestive system in order to study the consequences on subsequent food intake. In general, all of these studies reported that the enteral administration (stomach, duodenum, etc.) of nutrients leads to a reduction in subsequent intake, which has been interpreted as evidence of its satiating effect (Kohn 1951; Berkun et al. 1952; Glick and Modan 1977; Novin et al. 1979; Canbely and Koopmans 1984; Chapman et al. 1999; Reidelberger et al. 2003; Cox et al. 2004).

However, some authors have questioned this interpretation. Thus, animal experiments have demonstrated that under certain circumstances, although not always (Liebling et al. 1975; Tordoff and Friedman et al. 1986; Sclafani and Nissenbaum 1988; Lucas and Sclafani 1989; see Discussion below), the enteral administration of foods in association with a gustatory stimulus produces

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a strong rejection of the latter at subsequent presentations. This finding suggests that the reduction in intake after enteral administration of nutrients may result from their aversive effects rather than their satiating effects (Deutsch et al. 1976; Puerto et al. 1976a,b; Ramirez et al. 1997; Zafra 2000). These negative effects of the intragastric administration of nutrients have also been noted in the clinical setting. Thus, numerous studies have shown enteral feeding to be associated with various adverse effects (gastrointestinal discomfort, abdominal pain, irritation, fullness, flatulence, constipation, abdominal distension, bloating, cramping, vomiting, nausea, diarrhea, and ulcers, among others) that can even preclude this type of feeding for many patients (Heymsfield et al. 1979; Henderson et al. 1992; Elia 1994; Bengmark 1998; Jolliet et al. 1999; Whelan et al. 2002).

If the enteral administration of nutrients (obviating cephalic stimulation) can generate these noxious effects, it is of interest to identify the mechanisms involved in order to minimize the adverse consequences associated with artificial nutrition, for example, by acting on the neural centers responsible for processing visceral information. Information on substances present in the gastrointestinal tract can reach the brain by at least two pathways: the neural pathway, mainly mediated by the vagus nerve, allows rapid detection of the visceral stimuli (Chernigovskii 1962; Mei 1983; Smith 1983; Arnedo et al. 1993; Joyner et al. 1993; Sengupta and Gebhart 1994; Woods et al. 1998; Yamamoto and Sawa 2000; Schwartz 2000; Cox et al. 2004), whereas the humoral pathway involves a slower visceral processing (Coil and Norgren 1981; Smith 1983; Ossenkopp and Giugno 1985; Arnedo et al. 1990; Agüero et al. 1993b; Woods et al. 1998; Yamamoto and Sawa 2000). In the latter case, when signals are processed through the blood flow system, structures such as the area postrema, an important chemoreceptor zone, or its relay, the parabrachial complex, can form part of the circuit by which the visceral information is transmitted (Wang and Borison 1951; Ritter et al. 1980; Coil and Norgren 1981; Ossenkopp and Giugno 1985; Agüero et al. 1993b).

Within the parabrachial complex, the lateral parabrachial nucleus (LPB) has been specifically implicated in different intake-related processes, including food intake (Trifunovic and Reilly 2001; Zafra et al. 2002; Wilson et al. 2003), water intake (Edwards and Johnson 1991; Menani et al. 1996; De Gobbi et al. 2001; Tanaka et al. 2004), and taste aversions induced by toxic or noxious substances (Agüero et al. 1993a,b; Cubero and Puerto 2000; Chambers and Wang 2004).

With this background, the present work was designed to determine whether LPB lesions can block the negative effect of the intragastric administration of nutrients, usually observed (Deutsch et al. 1976; Puerto et al. 1976b; Ramirez et al. 1997; Zafra 2000) as an association with and rejection of previously presented flavor in two experimental situations: when the intragastric administration of nutrients is carried out immediately after presentation of the flavor stimulus (experiment 1), or when it is delayed (experiment 2). A second aim, given the participation of the LPB in processes related to food and water intake, was to determine the impact of LPB lesion and intragastric nutrient administration on subsequent food and water intake. Part of the present study has been reported in abstract form (Zafra et al. 2000).

Materials and methods

Animals

Experiment 1 used 20 male Wistar rats (260–320 g each) supplied from the breeding colony at the University of Granada, which were randomly assigned to one of two groups, an LPB-lesioned group ($n = 10$) and a control sham-lesioned group ($n = 10$). Experiment 2 used 18 male Wistar rats (290–315 g each), also randomly assigned to an LPB-lesioned group ($n = 11$) and a control sham-lesioned group ($n = 7$). Subjects were individually housed in $30 \times 15 \times 30$ cm cages that also served as training chambers during the experiment. The sides of the cages were black and opaque, and the front and back sides were transparent. The front side had two 1.6 cm holes at the same distance from the centre 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 by which flavors and water were delivered. The room was maintained on a 12:12-h light/dark cycle at 22 ± 1°C. The lights were on from 9:00 am to 9:00 pm, the period when experimental tests were conducted. Before undergoing surgery, the animals were allowed a 5–6 day adaptation period, during which time they remained in their cages and received food and water *ad libitum*. Animal protocols were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the Animal Care Guidelines established by Spanish Royal Law 223/1988.

Surgical procedure

Electrolytic lesions of the lateral parabrachial area (LPB). For the surgical operation, the rats were deeply anesthetized with an i.p. injection of sodium pentothal (50 mg/Kg, Lab. Abbot, Spain). They were placed in a stereotaxic apparatus (Stoeling Co. Stereotaxic Instruments 51.600), and an electrode was inserted into the area corresponding to the anteroposterior, lateral and vertical coordinates (A-P: − 1.4, L: ± 2.3, V: − 2.4), obtained from Pellegrino et al. (1979).

Cathodic current of 1 mA was applied bilaterally for 25 s using a model DCML-5 lesion maker (Grass Instruments Corp., Quincy, MA, USA) with a stainless steel monopolar electrode approximately 200 μm in diameter insulated to the last 0.5 mm. All of the above steps were also followed for the sham-lesioned control group except that the vertical coordinate was − 2.0 mm and no current was applied.

Intragastric catheters. An intragastric catheter was implanted using a modified version of the procedure developed by Deutsch and Koopmans (1973). In brief, a silastic tube (Silastic, Silastic-Medical Grade Tubing, Dow Corning Corp., Michigan, USA) was implanted into the cardiac portion of the stomach and routed through the abdominal muscle wall and under the skin to the back of the neck. Stitching was made as appropriate to help close the wounds, and, both the lesioned and control animals were given an intra-muscular 0.1 cc dose of penicillin (1000000 IU, Penilevel. Lab. Ern, Barcelona, Spain) in order to avoid the risk of infection.

Behavioral procedure

A period of 12–15 days was allowed for postoperative recovery. The subjects were then given 5 days of training, when they were placed on a daily schedule of access to tap water from a graduated burette for 10 min. Immediately afterwards, the animals were given solid food (Panlab, S.L. Barcelona), whose remains were withdrawn before starting the next training session. Both the food and the water intake were measured on the last three training days. The experiments began after the 5-day pre-training period. In experiment 1, the rats were offered a flavored solution (0.5% vanilla; McCormick Co. Inc., San Francisco, CA) for 7 min, immediately followed by the intragastric administration of 10 cc of a liquid diet (Ideal Evaporated whole milk, diluted 50% with water; Nestlé, Barcelona) at a rate of 1.6 ml/1 min; 100 ml of this liquid diet contained 5.75 g of carbohydrate, 3.93 g of fat, and 3.93 g of protein (total energy: 74.37 Kcal). Experiment 2 comprised three distinct phases (a, b, and c) of 6, 3 and 6 days, respectively. The procedure was similar to that for experiment 1 except that in the first part (experiment 2a), after intake of the flavor (0.5% vanilla; McCormick Co. Inc., San Francisco, CA) for 7 min there was a 15 min delay before the intragastric administration of 10 cc of the liquid diet. Experiment 2b was similar to 2a except that the flavored solution offered before the intragastric injections (vanilla) was replaced with water in order to analyze the specificity of the effect. In experiment 2c, the vanilla was again presented but, similar to experiment 1, there was no delay before the intragastric administration of nutrients. In both experiments (experiments 1 and 2), 20 g of solid food was offered 60 min after the intragastric administration, and its consumption was measured after 30, 60 and 90 min. After this period, food remains were withdrawn and access to water was permitted for 10 min. Immediately afterwards, sufficient solid food was again offered, and its remains were withdrawn at the end of the evening (14 h before the next experimental session). The body weight was measured daily throughout the experiment.

Histology

After completion of the behavioral procedure, animals in the lesioned group were deeply anesthetized with an overdose of sodium pentothal (100 mg/Kg) and intracardially perfused with isotonic saline followed by 10% formaldehyde (Formaldehído. Probus, S.A. Badalona). The brains were extracted and stored in formaldehyde for at least one week. Serial coronal sections were cut and stained with cresyl violet. The extent of lesions was examined under a light microscope.

Statistical analyses

Results were analyzed by two-way analysis of variance with the use of Statistica version 5.1 (from Statsoft, Tulsa, USA). Differences were considered significant at $P < 0.05$.

Results

Experiment 1

During experiment 1, the catheter became detached from two animals of the lesioned group and one of the control group, so that the analysis only included data from eight lesioned and nine control animals. During the three days before the experiment, the lesioned-group showed a higher food intake compared with the control group [$F(2,30) = 2.904$; $p < 0.029$] but no significant difference in water intake between the groups was observed [$F(1,15) = 0.51$; $p < 0.48$]. During the experiment, there were no differences between groups in consumption of the flavored solution presented before the intragastric administration of nutrients (Figure 1A; $F(1.15) = 2.19$, $P < 0.159$). There were no differences between groups in the intake of solid food at any measurement time (at 30 min [$F(1,15) = 1.18$, $P < 0.29$], 60 min (Figure 1B; $F(1,15) = 3.13$, $P < 0.09$], or 90 min $F(1.15) = 2.29$, $P < 0.15$]). However, there were significant differences in water intake at the end of the experimental sessions, with a higher intake by controls [Figure 1C; $F(1,15) = 9.57$, $P < 0.007$]. No significant differences in body weight were found between groups either on the day of the surgery [$F(1,15) = 0.659$, $P < 0.429$] or during the experiment [$F(1,15) = 2.15$, $P < 0.163$].

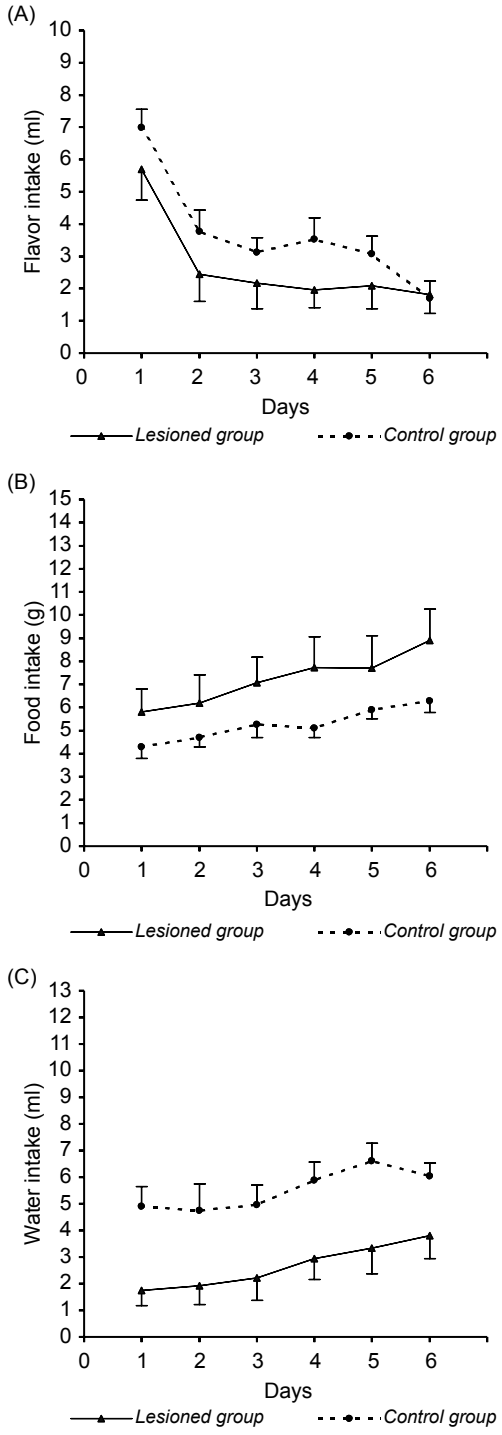

Figure 1. Mean amounts of the flavored solution (A), standard rat chow (B) and water (C) consumed by the subjects in experiment 1. In this experiment, the intragastric administration of nutrients was immediately after intake of the flavored solution. The data for solid food intake (B) correspond to the intake by animals measured at 60 min after its presentation.

Experiment 2

During experiment 2, two animals from the lesioned group died, so that the analysis only included data from nine lesioned animals. During the three days before the experiment, the groups did not significantly differ in their daily food intake, although there was a tendency towards a significant difference [$F(1,14) = 3.33$; $p < 0.08$], or in their water intake [$F(1,14) = 1.06$; $p < 0.32$]. In experiment 2a, the interaction between factors (group and days) was highly significant in relation to the intake of flavor (vanilla) presented at the start of the sessions, with a higher intake by the lesioned-group [Figure 2A; $F(5,70) = 7.979$, $P < 0.001$]. Subsequent analyses showed that this difference was already significant on day 5 [one-way ANOVA, day 5: $F(1,14) = 6.370$, $P < 0.024$; day 6: $F(1,14) = 15.50$, $P < 0.001$]. The lesioned-group also consumed significantly more solid food at all measurement times: at 30 min [$F(1,14) = 6.237$, $P < 0.025$], 60 min [Figure 2B; $F(1,14) = 8.137$, $P < 0.012$], and 90 min [$F(1,14) = 6.602$, $P < 0.022$]. Finally, analysis of the water intake (Figure 2C) showed that the days variable [$F(5,70) = 3.375$, $P < 0.008$] and the interaction between groups and days [$F(5,70) = 5.515$, $P < 0.001$] were significant, with a higher intake by controls.

In experiment 2b, there were no significant differences between the groups in water intake before the intragastric administration of nutrients [Figure 3A; $F(1,14) = 0.194$, $P < 0.66$] or at the end of the sessions [Figure 3C; $F(1,14) = 1.878$, $P < 0.19$]. The solid food intake of the lesioned-group was higher than that of the control-group, although only at the 30-min [significant interaction, $F(2,28) = 3.854$, $P < 0.033$] and 60-min [Figure 3B; $F(1,14) = 5.63$, $P < 0.032$] measurements. At 90 min, there was a tendency to a significant difference [$F(1,14) = 4.03$, $P < 0.06$].

On the other hand, comparison between the intake of the flavored solution (vanilla) in experiment 2a with the intake of water in experiment 2b showed a strong interaction between the last two days of experiment 2a and the first two of experiment 2b [$F(3,42) = 14,26$ $P < 0.001$]. When these data were subsequently analyzed in each of the groups, these differences were found to be significant for both the control [$F(3,18) = 37.47$, $P < 0.001$] and the experimental [$F(3,24) = 3.41$, $P < 0.03$] group. These results appear to suggest that whereas the control group perceives the flavor in experiment 2a as aversive, the lesioned group shows a preference for it.

In experiment 2c, analysis of variance showed that the flavor (vanilla) intake of the lesioned-group was higher than that of the control-group [Figure 4A; $F(1,14) = 8.12$, $P < 0.012$], and that there was a significant interaction between group and days [$F(5,70) = 2.92$, $P < 0.018$]. The lesioned group also showed a significantly higher intake of solid food at the three measurement times (Figure 4B): at 30 min [$F(1,14) = 4.765$, $P < 0.046$], 60 min [$F(1,14) = 5.63$, $P < 0.032$], and 90 min [$F(1,14) = 12.54$, $P < 0.003$]. In contrast, the intake of water at the

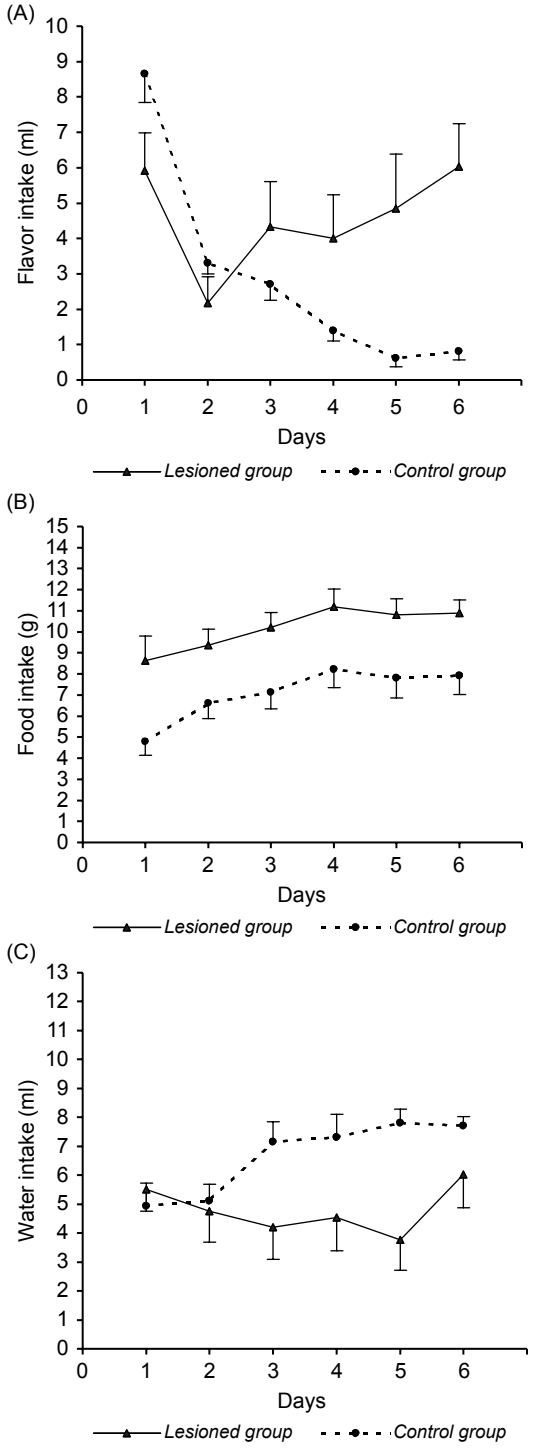

Figure 2. Mean amounts of the flavored solution (A), standard rat chow (B) and water (C) consumed by the subjects in experiment 2a. The data for standard rat chow (B) correspond to the intake by animals measured at 60 min after its presentation. In this experiment, the intragastric nutrient administration was 15 min after intake of the gustatory stimulus.

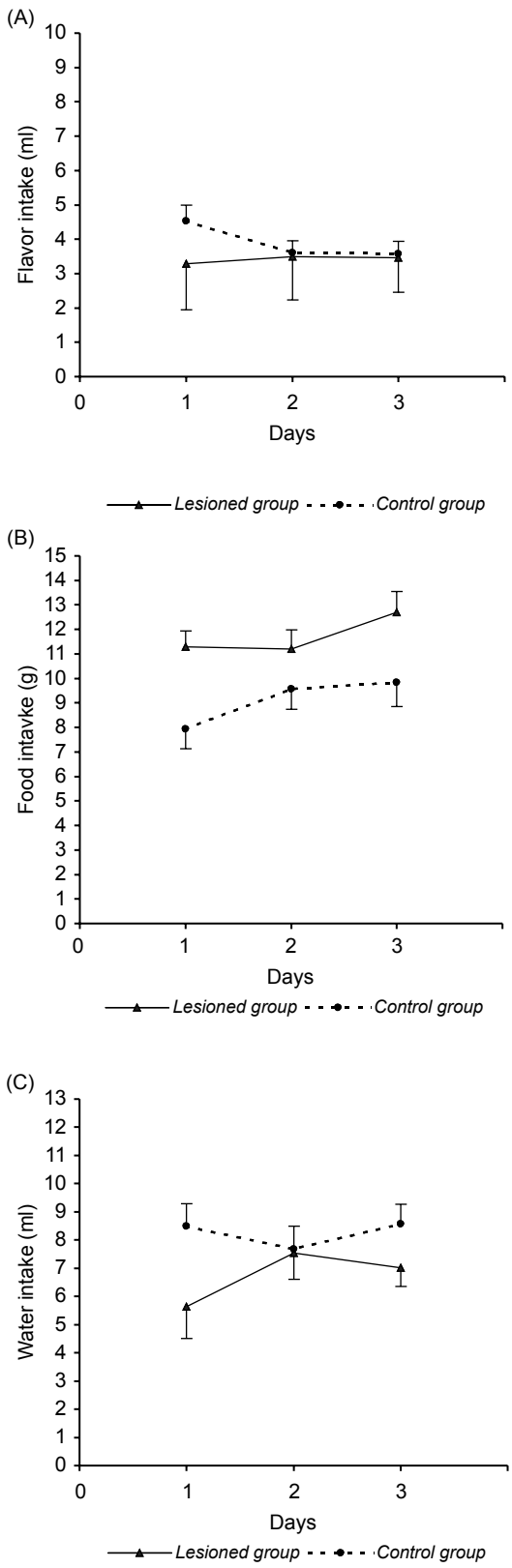

Figure 3. Mean amounts of water presented prior to the intragastric nutrient administration (A) and after withdrawal of the solid food (C) consumed by the subjects in experiment 2a. Graph B depicts the mean amounts of standard rat chow consumed by experiment 2b subjects measured at 60 min after its presentation. In this experiment, the intragastric nutrient administration was 15 min after intake of the gustatory stimulus (water).

end of the sessions was significantly higher in the control-group [Figure 4C; $F(1,14) = 20.46$, $P < 0.001$]. No significant differences in body weight were found between the groups either on the day of the surgery [$F(1,14) = 1.51$, $P < 0.238$] or during

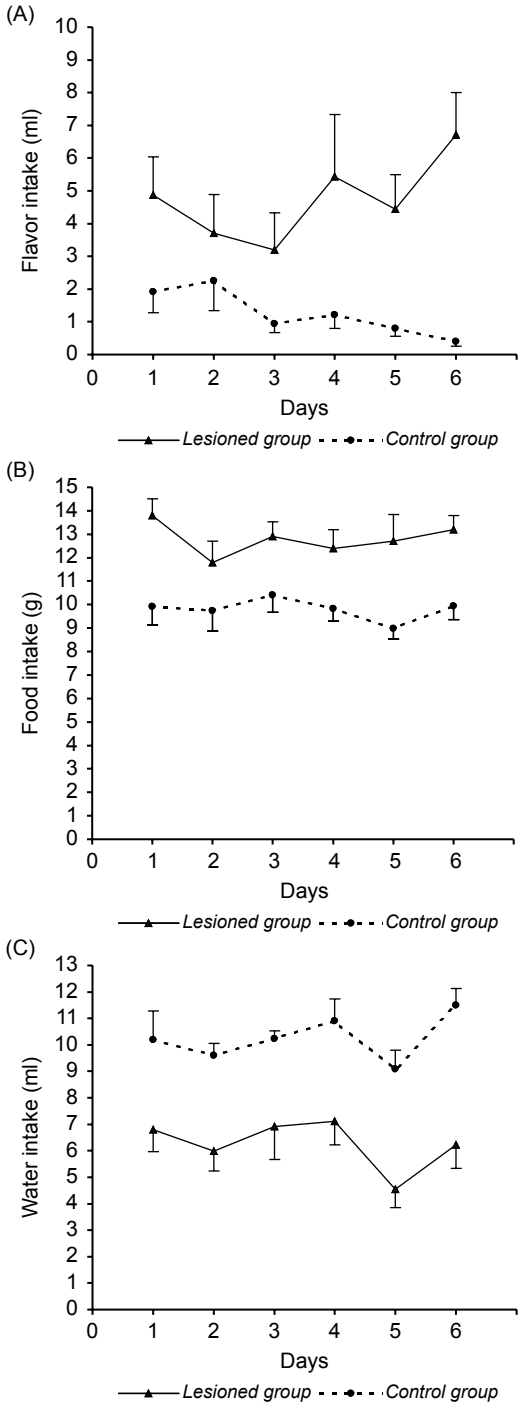

Figure 4. Mean amounts of the flavored solution (A), standard rat chow (B) and water (C) consumed by the subjects in experiment 2c. The data for standard rat chow (B) correspond to the intake by animals measured at 60 min after its presentation. In this experiment, the intragastric administration of nutrients was immediately after intake of the flavored solution.

the experiment (experiment 2a) [$F(1,14) = 0.311$, $P < 0.58$], 2b [$F(1,14) = 0.0055$, $P < 0.94$], or 2c [$F(1,14) = 0.203$, $P < 0.65$].

Histological results

Histological analysis of the coronal sections revealed appropriately localized lesions. Figure 5 shows a microphotograph that illustrates the electrolytic lesions produced in these animals.

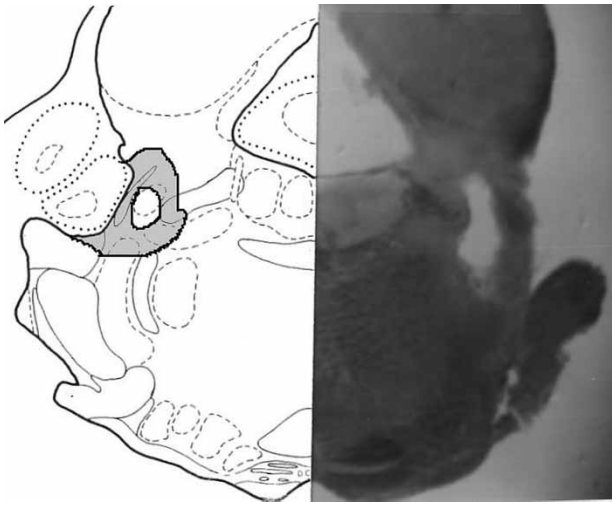

Figure 5. Representative photomicrographs of coronal sections showing electrolytic lesions of the LPB. The schematic illustration was adapted from the Pellegrino, Pelley, and Cushman atlas [33] and represent the largest (shown by grey area) and smallest (central white area) LPB lesions. The smallest area shown represents the overlap zone between animals.

Discussion

Our experiments with neurologically intact subjects demonstrated that the intragastric administration of nutrients produces an aversive effect that is manifested in the rejection of associated gustatory stimuli, as previously reported (Deutsch et al. 1976; Puerto et al. 1976b; Zafra 2000). We also observed this rejection in naïve LPB-lesioned animals when the visceral stimulus was administered immediately after presentation of the flavor (experiment 1) but not when there was a delay between these presentations (experiment 2). As shown in Figure 1A, the flavor avoidance was similar in both groups (lesioned and controls) in experiment 1 and increased over time with repeated gustatory stimulus–visceral stimulus associations. In contrast, when there was a delay between the presentation of the flavor and the intragastric nutrient administration (experiment 2), LPB-lesioned animals showed no rejection of the gustatory stimulus (Figure 2A). In the latter experiment, the flavor intake was much higher than that observed in the neurologically intact animals and, at the end of the six-day period, the intake was similar to that on the first experimental day before any intragastric administration.

These data suggest that the LPB lesion blocks the aversive component of intragastrically administered nutrients when their presentation is delayed with respect to presentation of the flavored solution.

Interestingly, the results obtained in experiment 2a are similar to those obtained in neurologically intact animals when instead of natural foods they are intragastrically administered with pre-digested foods

(Zafra et al. 2005, submitted), which are perceived by the subjects as rewarding (Puerto et al. 1976a; Zafra et al. 2002). Furthermore, these results appear to support the suggestion that the LPB lesion in the delayed paradigm not only removes the aversive component of the intragastrically administered natural foods but also converts them into preferred foods, as observed when vanilla (experiment 2a) and water (experiment 2b) intakes were compared on four successive days.

Our data support the proposal of two different mechanisms that explain the consequences of intragastrically administered nutrients (Agüero et al. 1993a,b; Mediavilla et al. 2000, 2005). One is independent of the LPB, given that the lesioned animals and controls both show aversion to the intragastrically administered nutrients by rejecting the associated gustatory stimulus. The other mechanism participates after an inter-stimulus delay, and an intact LPB nucleus appears to be essential. Thus, when the intragastric administration of nutrients is delayed, the negative effect is manifest in controls but not in LPB-lesioned animals, probably due to the disruption of blood-borne mediating digestive signals.

This anatomical dissociation of two visceral processing systems is consistent with data related to the regulation of food intake. Thus, it is accepted that nutrient intake is controlled by a dual neurobiological substrate that informs the brain about the nutritional state of the subject. The first mechanism, responsible for short-term satiety or satiation, is a rapid action mechanism of an essentially vagal nature involved in the cessation of consumption behavior (Gonzalez and Deutsch 1981; Joyner et al. 1993; Phillips and Powley 1998; Cox et al. 2004). The second mechanism, with a more delayed action, participates in long-term satiety and depends on post-absorptive effects such as the availability of nutrients and the rate of their utilization or storage in adipose tissue (Smith 1983; Woods et al. 1998).

The biological process underlying the interruption by this extensive lateral parabrachial region lesion of the aversive effect in the delayed task is not completely clear. However, it has been demonstrated that there are two clearly distinguishable dimensions in the processing of different sensory systems, i.e. a sensory-discriminative and an affective-emotional dimension (Schnitzler and Ploner 2000; Rojas-Corrales et al. 2002; Gao et al. 2004; Sewards 2004; Hajnal and Norgren 2005). Data published relatively recently suggest that the parabrachial area may participate in the processing of affective/aversive aspects of certain stimuli (Bechara et al. 1993; Bernard et al. 1994; Bester et al. 2000; Bourgeais et al. 2001; Gauriau and Bernard 2001; Sewards 2004). It can, therefore, be hypothesized that, in the present experiment, lesions confined to this area would have blocked the affective-aversive component of the visceral stimulus, thereby inducing a change in the valence of the nutrients.

In the second part of experiment 2 (experiment 2b), when water, which is not a novel stimulus, was presented instead of the flavor (vanilla), the negative effects of the intragastric nutrient administration were not transferred and, therefore, the behavior of the two groups equalized (Figure 3A). These data suggest that preference (lesioned group) and rejection (control group) were specific to the gustatory stimulus. Interestingly, the differences between the two groups returned when the vanilla was again presented in the third part (experiment 2c), despite the fact that the gustatory stimuli were presented without delay, as in experiment 1.

Thus, the results of experiment 2c (Figure 4A) markedly contrast with those of experiment 1, in which the lesioned animals showed a strong rejection of the flavor. A possible explanation of this difference may be that, because the gustatory stimulus presented in experiments 2c and 2a were the same, the lesioned animals in experiment 2a learned that vanilla did not have aversive effects, so that they did not associate the two stimuli when the delay was eliminated (in 2c); once the animals established a visceral-gustatory association they did not modify it, at least with respect to the gustatory stimulus. None of the groups showed aversion to water when it was presented prior to the intragastric administration (experiment 2b), probably because its intake at the end of the sessions had no negative effects and, above all, because of their previous experience of water as an innocuous product.

The results obtained in this study appear to contradict published reports that the intragastric administration of nutrients induced the development of flavor preferences (Liebling et al. 1975; Tordoff and Friedman 1986; Sclafani and Nissenbaum 1988; Lucas and Sclafani 1989). We believe that a key factor may be the cephalic/neural phase and the utilization or not of foods subjected to the cephalic-phase of digestion, a set of endocrine and autonomic responses of the digestive system that result from stimulation of sensory systems at the cephalic level, especially in the oropharyngeal cavity (Giduck et al. 1987; Pavlov 1910; Teff 2000). Hence, when the cephalic phase is deliberately obviated, as in the present study and other investigations (Puerto et al. 1976b; Puerto 1977; Zafra 2000), the intragastric administration of nutrients induces aversive effects, perhaps because the gastrointestinal tract is in a physiologically inadequate condition for the reception, digestion and absorption of the food at the same time as initiating its immediate transformation (Giduck et al. 1987; Pavlov 1910). Numerous studies have shown that when this phase is absent, directly administering nutrients into the gastric cavity or gut, major physiological and behavioral dysfunctions are observed (Pavlov 1910; Molina et al. 1977; Giduck et al. 1987; Kaplan et al. 1993; Yamashita et al. 1993; Friedman et al. 1996; Horn et al. 1996;

Ramirez et al. 1997; Teff 2000). Conversely, when this neuroendocrine process is present in some form, as in studies by our group (Puerto et al. 1976a,b; Puerto 1977; Zafra 2000; Zafra et al. 2002) and other authors (Liebling et al. 1975; Tordoff and Friedman 1986; Ackroff and Sclafani 1994; Ramirez 1994), the intragastric administration of nutrients is reinforcing.

It is our understanding that involvement of the cephalic phase and induction of flavor preferences after the intragastric administration of nutrients can be achieved by utilizing cephalic/pre-digested foods from donor animals (Puerto et al. 1976a,b; Puerto 1977; Zafra 2000; Zafra et al. 2002) or by the utilization of gustatory stimuli to which saccharin has been added (Liebling et al. 1975; Lucas and Sclafani 1989; Ramirez 1994; Sclafani et al. 1996; Azzara and Sclafani 1998; Sclafani et al. 1999; Sclafani 2002; Ackroff et al. 2005). It has been demonstrated that saccharin can induce some responses proper to the cephalic phase of digestion (Berthoud et al. 1980; Ionescu et al. 1988; Tordoff and Friedman 1989). Furthermore, some studies have reported that the intragastric administration of "natural" nutrients is only effective in establishing conditioned flavor preferences or increasing fluid intake when saccharin is used as the gustatory stimulus and not when the stimulus is simply flavored water (Lucas and Sclafani 1989; Ackroff and Sclafani 1994; Ramirez 1994). Likewise, this procedure for inducing gustatory preferences can be facilitated by the fact that long time periods are often used, usually 20–23 h and over many days, and solid food can even be available *ad libitum*. These orally ingested solid foods can also trigger the cephalic phase, therefore, the stomach will be availed of the appropriate secretions for reception of the nutrients, both those consumed via the oropharyngeal cavity and those enterally administered with the intake of the gustatory stimulus. Finally, the induction of flavor preferences may also be facilitated (following the above procedures) by the fact that nutrients used in intragastric administration are often highly diluted substances (2.7–14.5% fats, 6–16% maltodextrin, glucose, maltose and fructose, 0.5–32% polycose) with a very low caloric content (at each gustatory stimulus intake episode) such that major digestive secretions are not required. This is especially true when nutrient digestion is favored by the frequent use of partially hydrolyzed products such as polycose or emulsion foods (fats), i.e. foods that have been previously broken down. In contrast, studies by our group have used complex natural foods (evaporated whole milk) as visceral stimuli, and the absence of the cephalic phase under these conditions can induce a major noxious effect and the aversive effect that we have repeatedly observed (also observed in humans, as mentioned above).

In experiment 2, the neurologically intact animals consumed less solid food after the intragastric nutrient administration compared with the LPB-lesioned animals (Figures 2B and 3B). This reduction cannot be attributed to a lesser previous intake of liquid, because in the first four days of experiment 2a, when the intake of flavored solution did not significantly differ between the two groups, there was a significant difference in the intake of solid food (Figure 2B). This interpretation is supported by the results of experiment 2b, when the previous water intake was similar (Figure 3A) but the difference in solid food intake persisted for most of the days (Figure 3B). Therefore, the reduction in consumption shown by non-lesioned animals does not appear to be related to the amount of previous liquid intake.

In our study, there was a difference in food intake after the intragastric administration of nutrients in experiment 2, when the lesioned animals were unable to associate the gustatory and visceral stimuli, but not in experiment 1 (although there was a tendency for the lesioned group to consume more food versus the controls). In experiment 1, in which the learning of the lesioned and intact animals was established with the same efficacy, there was an inhibitory effect on subsequent intake with no significant differences between the groups. Therefore, under normal circumstances, the effect on intake may be determined both by the intragastric nutrient administration itself and by the ability of the animal to attribute the supposed discomfort that the administration produces. Thus, in experiment 2, the lesioned animals did not appear to attribute this supposed negative effect to the previous gustatory stimulus and there was no inhibition of the subsequent food intake, unlike in the controls.

However, a complementary explanation is also possible. The increase in food intake after enteral nutrient administration shown by the lesioned animals in our experiment 2 may also be due in part to the hyperphagia produced by the LPB lesion. This is suggested by the data obtained during the training period in our experiment, when the food intake tended to be higher in the lesioned versus intact animals (although the differences in experiment 2 did not reach significance). These findings are consistent with reports by other authors. Thus, Nagai et al. (1987) demonstrated that lesion of the dorsolateral parabrachial triggered an increase in food intake and, therefore, a significant increase in body weight.

Finally, in consonance with previous studies (Ohman and Johnson 1986; Edwards and Johnson 1991; Menani et al. 1996), the present experiments indicate that LPB lesion does not itself affect water intake, given the absence of significant differences between the groups during the training period. Nevertheless, there were differences in this behavior after the intragastric administration of nutrients. In all cases, the lesioned animals showed a smaller intake of the water offered at the end of the experimental

sessions (Figures. 1C and 2C). In experiment 2, this may be thought to be due to a greater consumption of liquid by the lesioned animals during the learning sessions. However, this interpretation is unlikely because the differences persisted at the end of the learning sessions in experiment 1, when both groups ingested similar amounts of liquid at the beginning of the sessions (Figure 1C).

The data presented in the present study may have clinical relevance, especially in the setting of artificial nutrition. Enteral nutrition is frequently accompanied by serious adverse effects that can even lead its contraindication (Heymsfield et al. 1979; Henderson et al. 1992; Elia 1994; Bengmark 1998; Jolliet et al. 1999; Whelan et al. 2002). According to our results in animals, these negative effects of enteral nutrition in humans can be palliated by intervention (e.g. pharmacological treatment) on the activity of the neural centers involved in visceral processing.

In conclusion, the present work shows that LPB lesion disrupts the aversive component of the intragastric administration of nutrients, as demonstrated by the inability to associate this component with a previously presented flavored solution, when there was a delay between the stimulus presentation and the nutrient administration. In fact, an induced-flavor preference (versus water) was observed in the late trials of the learning task. It was also demonstrated that LPB lesions only lead to a greater consumption of solid food presented after intragastric nutrient administration in animals in which the aversive process has not developed. However, LPB lesions produced an inhibitory effect on water intake in both cases.

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