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Research report

Satiation and re-intake after partial withdrawal of gastric food contents: A dissociation effect in external lateral parabrachial lesioned rats

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ABSTRACT

Sensory information from the gastrointestinal system can be transmitted to the brain through the vagus nerve, the intermediate-caudal region of the nucleus of the solitary tract (NST), and various subnuclei of the parabrachial complex, notably the external lateral subnucleus (LPBe). The objective of the present study was to examine the relevance of this subnucleus in satiation and food reintake after gastrointestinal food removal. LPBe-lesioned animals were subjected to a re-intake task following the partial withdrawal of gastric food contents shortly after satiation. Lesioned and control animals ingested a similar amount of the initial liquid meal. However, after withdrawal of one-third of the food consumed, LPBe-lesioned rats were not able to compensate for the deficit created, and their re-intake of food was significantly lower than the amount withdrawn after the satiating meal. In contrast, the food re-intake of control animals was similar to the amount withdrawn. Hence, the LPBe does not appear to be critical in the satiation process under the present experimental conditions. However, the LPBe may be part of a system that is essential in rapid visceral adjustments related to short-term food intake, as also shown in other gastrointestinal regulatory behaviors that require immediate processing of visceral sensory information.

1. Introduction

Food intake is a complex behavioral process controlled by specific peripheral neural systems, blood-borne factors, and various brain structures (Hamr et al., 2015). There is a degree of consensus that the regulation of different nutritional processes requires initial volumetric and chemical information that is generated in the upper gastrointestinal tract in response to the food being consumed (Eisen et al., 2001; Folgueira et al., 2014; Phillips and Powley, 1996, 1998; Sengupta and Gebhart, 1994).

Part of this information can be transmitted to the brain *via* the sensory component of the vagus nerve (Altschuler et al., 1989; Sengupta and Gebhart, 1994). The intermediate-caudal region of the nucleus of the solitary tract (NSTic), its first main central relay (Altschuler et al., 1989; Shapiro and Miselis, 1985), projects to several parts of the lateral division of the pontine parabrachial complex, including the external lateral parabrachial subnucleus (LPBe) (Herbert et al., 1990).

A role for the LPBe has been proposed in various food regulatory mechanisms in which the vagal-NSTic axis is known to be important. Thus, pharmacological or endocrine agents that stimulate or inhibit food intake were found to activate both the intermediate-caudal region of the NST (Day et al., 1994) and the LPBe, among other brain regions (Horn and Friedman, 1998a, 1998b; Li and Rowland, 1995, 1996; Rowland et al., 1997; Yang et al., 2004). Conversely, wide lesions of the LPB area, which likely includes the LPBe, block the effects of these agents on intake (Becskei et al., 2007; Calingasan and Ritter, 1993; Trifunovic and Reilly, 2001). Both the neuronal activation and/or intake effects can also be abolished or attenuated by truncal vagotomy or by perivagal capsaicin treatment of the vagus nerve (Horn et al., 2001; Ladenheim and Ritter, 1991; Li and Rowland, 1995; Ritter et al., 1994; Smith et al., 1981; Yang et al., 2004).

It has also been shown that LPB cell activity (including the external subnucleus), is sensitive to electrical stimulation of the

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vagus nerve (Gieroba and Blessing, 1994; Saleh and Cechetto, 1996) and NSTic (Suemori et al., 1994). Studies with c-fos techniques or single-unit recordings have also demonstrated activation of the LPB/LPBe after gastric distension (Baird et al., 2001), free feeding (Yamamoto et al., 1994), and infusion of nutrients into the stomach (Emond et al., 2001; Kobashi et al., 1993; Yamamoto and Sawa, 2000a, 2000b) or duodenum (Wang et al., 1992).This activation can be abolished or significantly attenuated by vagus nerve lesions (Yamamoto and Sawa, 2000a).These data suggest that the LPBe may be part of the afferent pathway involved in the processing of vagal-brain information associated with meal size-related signals.

It has also been demonstrated that an intact vagus nerve (Zafra et al., 2006, 2007), NSTic (Mediavilla et al., 2011), and LPBe (Mediavilla et al., 2000; Zafra et al., 2002) are essential for taste discrimination learning tasks that require rapid processing of the intragastric administration of aversive substances (Mediavilla et al., 2000; Zafra et al., 2006) or rewarding nutrients (Zafra et al., 2002, 2007), while concurrent electrical stimulation of the LPBe (Simon et al., 2007) or optogenetic activation of the PVH-Parabrachial axis (Garfield et al., 2015) was found to induce conditioned place pReferences

With this background, the objective of the present study was to examine the relevance of the LPBe subnucleus in short-term food intake tasks presumed to require rapid processing of gastrointestinal information, in which the vagus nerve appears to participate (Phillips and Powley, 1998; Zafra et al., 2003, 2006, 2007). For this purpose, LPBe-lesioned animals were subjected to a re-intake test, in which the volume of an initial liquid meal consumed by animals up to satiation was measured, part of the gastric contents was then pumped out, and the subsequent re-intake of food was compared with the volume withdrawn.

2. Materials and methods

2.1. Subjects

This study used 24 naïve adult male Wistar rats (290–315 g at the beginning of the experiment) from a breeding colony of the University of Granada. Animals were randomly assigned to an LPBe-lesioned group or sham-lesioned (control) group (n = 12 in each). Upon arrival at our lab, animals were individually housed in 30 × 15 × 30 cm methacrylate cages with free access to water and pelleted stock diet (Panlab, S.L. Barcelona). The room temperature was maintained at 22 ± 1 °C with 12-h light-dark periods. All experimental procedures and surgical techniques took place during the light phase and were conducted in accordance with the Animal Care and Use Guidelines established by European Community Council Directive 86/609/CEE and approved by the Ethical Committee for Animal Experimentation of the University of Granada.

2.2. Surgical procedure

2.2.1. Lesions of the external lateral parabrachial subnucleus (LPBe)

Surgery was carried out under general anesthesia with sodium pentothal (50 mg/Kg, ip; sodium thiopental, Abbot Laboratories, Spain). Once animals were anesthetized, they were placed in a stereotaxic device (Stoelting Co. Stereotaxic 51.600), and a cathodic electric current (0.3 mA) was bilaterally applied for 10 s using a DCML-5 lesion-maker (Grass Instruments Corp., Quincy, Mass, USA). Electric current was supplied through a 00 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 LPBe were obtained (interaural references) from the Paxinos and Watson stereotaxic atlas (Paxinos and Watson, 1996): anterior/posterior (AP) = −0.16 mm; lateral (L) = ± 2.5 mm; and ventral (V) = +3.0 mm.

All of the above steps were followed for the sham lesion control group except that a vertical coordinate of + 4.0 mm was used and no current was applied.

2.2.2. Intragastric catheter

After the brain surgery, an intragastric catheter was implanted using a modified version of the procedure developed by Deutsch and Koopmans (1973). In brief, a silastic tube (ID = 1 mm; OD = 2 mm; Dicoinsa, S.L., Barcelona, Spain) was implanted into the cardiac portion of the stomach at the greater curvature, routed through the abdominal muscle wall, and placed under the skin for exteriorization at the back of the neck. Stitching was performed as needed to help close the wounds, and an intramuscular 0.1 cc dose of penicillin (1,000,000 U; Penilevel Retard. Lab., Level, S.A. Barcelona) was administrated as prophylaxis against infection.

2.3. Behavioral procedure

Before the surgery, rats underwent a 24-h period of food and water deprivation followed by a 4-day adaptation period. On days 1 and 2 of this adaptation period, a liquid diet of chocolate-flavored milk (Puleva Food, S.L., Spain; 100 ml contains 12.2 g of carbohydrates, 2.2 g of fat, and 3 g of protein; total energy of 81 Kcal) was offered at 10:00 for 60 min and at 13:00 for 30 min. On days 3 and 4, this diet was offered only at 10:00 for 20 min. On the first three adaptation days, water (for 10 min) and solid food (7.5 g on days 1 and 2 and 10 g on day 3) were offered at the end of the morning. On day 4 of the adaptation period, solid food (pelleted stock diet) and water were available *ad libitum* after consumption of the liquid diet and for the next six days; the amount of food consumed was measured daily. The animals underwent surgery on day 3 of this six-day period. From the end of the morning on day 6, they were again deprived of access to food and water. The animals then underwent a 3-day pre-training period for re-adaptation to the liquid diet. On day 1 of this period, they were offered chocolate-flavored milk twice in the morning and once in the afternoon until satiation (5 min without consuming). On days 2 and 3, the diet was only offered once in the morning until satiation. On days 1 to 3, the animal was taken out of its cage after the intake session (on day 1 after the first morning session) and carefully handled in order to simulate the experimental situation; the animals were then offered water for 10 min (after the afternoon session on day 1) (Table 1).

2.4. Experiment 1A

Experiment 1A began the day after the 3-day post-surgery pretraining period. The session (in the morning) began by offering a burette with chocolate-flavored milk that the animals consumed until satiation (5 min without intake). The latency to intake initiation (intake latency) and the duration of the intake were recorded. After 5 min without consuming, animals were removed from their cage and one-third of the food ingested was withdrawn from the stomach, a procedure that usually took 0.5–1 min. Next, they were returned to the cage, where the liquid diet remained available *ad libitum* for 20 min, and their intake was quantified every 5 min.

To ensure adequate nourishment of the animals, the chocolate-flavored milk was presented again in the afternoon for 30 min (such that the deprivation period until the next morning session was 16 h).

2.5. Experiment 1B

Experiment 1 B was conducted on the next day with the same animals in order to confirm that the effects observed in experi-

Table 1
Time course of experiment.

	Food & water privation	ADAPTION PERIOD (PRESURGERY)						Surgery
DAYS	-7	-6	-5	-4	-3	-2	-1	0
		Chocolate-flavored milk 10:00 (60 min) 13:00 (30 min)	**Chocolate-flavored milk** 10:00 (60 min) 13:00 (30 min)	**Chocolate-flavored milk** 10:00 (30 min)	**Chocolate-flavored milk** 10:00 (30 min)	**Solid food & water** *Ad libitum*	**Solid food & water** *Ad libitum*	**Solid food & water** *Ad libitum*
		Water 14:00 (10 min)	**Water** 14:00 (10 min)	**Water** 11:00 (10 min)	**Solid food & water** *Ad libitum*			
		Solid food 14:10 (7,5 g)	Solid food 14:10 (7,5 g)	**Solid food** 11:10 (10 g)				

	RECOVERY PERIOD			PRETRAINING PERIOD			EXPERIMENTS 1A, 1B, 1C		
				Food & water privation			**1A**	**1B**	**1C**
DAYS	1	2	3	4	5	6	7	8	9
	Solid food & water *Ad libitum* 24h food intake	**Solid food & water** *Ad libitum* 48h food intake	**Solid food & water** *Ad libitum* 72h food intake	**Chocolate-flavored milk** 10:00 (satiation) 13:00 (satiation) 18:00 (satiation) **Water** 18:30 (10 min)	**Chocolate-flavored milk** 10:00 (satiation) **Water** 11:00 (10 min)	**Chocolate-flavored milk** 10:00 (satiation) **Water** 11:00 (10 min)	**Chocolate-flavored milk** (satiation) **1/3 gastric contents revomed** **Food intake** 5,10,15,20 min	**Chocolate-flavored milk** (satiation) **no gastric contents revomed** **Food intake** 5,10,15,20 min	**Chocolate-flavored milk** (satiation) **1/3 gastric contents revomed** **Food intake** 5,10,15,20 min

ment 1A were due to the food withdrawal from the stomach. In this experiment, the behavioral test was identical to that of experiment 1A except that no food was withdrawn from the stomach: after 5 min without consuming (satiation criterion), the animal was removed from its cage and underwent the same procedure as in experiment 1A except that no food was withdrawn from the stomach.

2.6. Experiment 1C

Transient effects can sometimes be produced by interruption of the vagal-parabrachial gastrointestinal information-processing pathway (Zafra et al., 2003; Agüera et al., submitted). Experiment 1C was designed to examine this possibility, being an exact replica of experiment 1A in the same animals but performed on the day after experiment 1B. The aim was to confirm the consistency of the results obtained in Experiment 1A.

2.7. Histology

After concluding the behavioral procedures, animals in the lesioned group were deeply anesthetized with a sodium pentothal overdose and intracardially perfused with isotonic saline followed by 10% formaldehyde (Formaldehyde. Probus, S.A. Badalona). The brains were removed and stored in formaldehyde for at least one week. They were then sectioned in the coronal plane at 40 μm on a cryostat (Microm HM550, MICROM International GmbH, Walldorf, Germany), and sections were mounted and stained with cresyl violet. These were examined under a light microscope (stereoscopic microscope UMZ-4F; Olympus, Tokyo, Japan) and microphotographed (PM-G; Olympus Optical, Tokyo, Japan) to determine the localization and extent of the injury, which can be facilitated by the utilization of electrolytic lesions. Fig. 1 depicts the results of the histological study.

2.8. Statistical analyses

Results were analyzed by three-way and one-way repeated measure ANOVAs. Statistica version 5.1 (Statsoft, Tulsa, USA) was used for the data analyses, and $p < 0.05$ was considered significant. Significant effects were analyzed by means of a post-hoc Tukey test. The re-intake was analyzed using percentage data (% meal size).

3. Results

Two animals were excluded from the control group, one for showing turning behavior and the other for catheter detachment. Hence, the statistical analyses only included the data from 22 animals (12 lesioned and 10 controls).

3.1. Food intake and body weight pre-surgery

Before surgery, the groups did not significantly differ in mean intake of daily chocolate-flavored milk [$F(1,20) = 0.096$, $p < 0.75$; two-way ANOVA] or solid food [$F(1,20) = 2.55$, $p < 0.125$; two-way ANOVA] or in mean body weight [$F(1,20) = 0.54$; $p < 0.47$; two-way ANOVA].

3.2. Food intake and body weight post-surgery

In the days after the surgery, the groups did not differ in mean intake of chocolate-flavored milk [$F(1,20) = 2,17$, $p < 0.156$; two-way ANOVA], intake of solid food [$F(1,20) = 1.68$, $p < 0.2$; two-way ANOVA], or body weight [$F(1,20) = 3.24$; $p < 0.086$; two-way

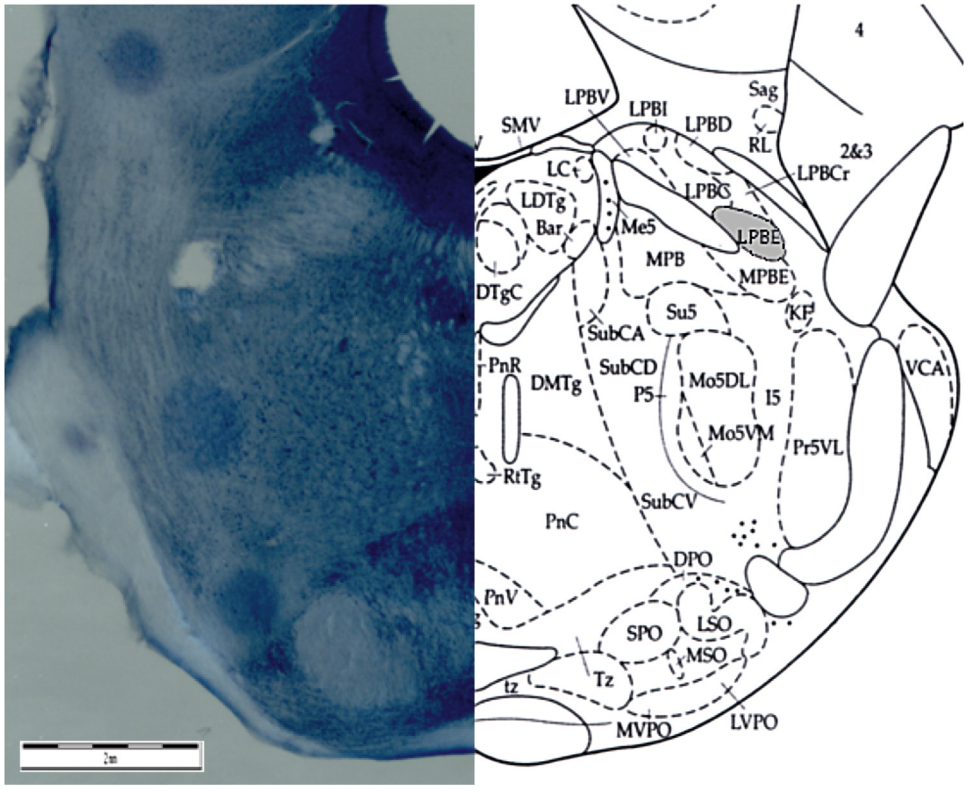

Fig. 1. Histological preparation stained with cresyl violet, showing the localization of the LPBe lesion in a representative animal of this experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ANOVA (body weight was maintained slightly higher in the LPBe-lesioned animals versus controls)].

3.3. Chocolate-flavored milk intake pre-extraction

One-way ANOVA showed that the groups did not significantly differ during any of three parts of the experiment (A, B, or C) in chocolate-flavored milk intake latency [1A: $F(1,20) = 0.62$; $p < 0.43$; 1B: $F(1,20) = 0.13$, $p < 0.71$; 1C: $F(1,20) = 0.068$, $p < 0.79$], in the duration of their intake [1A: $F(1,20) = 0.22$; $p < 0.63$; 1B: $F(1,20) = 0.44$, $p < 0.51$; 1C: $F(1,20) = 1.58$, $p < 0.22$], in their total intake before stomach content extraction [A: $F(1,20) = 1.72$; $p < 0.2$ (LPBe: 16.36 ml, Control: 14.81 ml); B: $F(1,20) = 2.19$; $p < 0.15$ (LPBe: 17.23 ml, Control: 15.45 ml); C: $F(1,20) = 1.45$, $p < 0.24$ (LPBe: 19.2 ml, Control: 17.62 ml)] or, therefore, in the amount intragastrically withdrawn (one-third of contents) after 5 min of satiation [A: $F(1,20) = 1.64$, $p < 0.21$ (LPBe: 5.42 ml, Control: 4.91 ml); B; C: $F(1,20) = 1.37$, $p < 0.25$ (LPBe: 6.35 ml, Control: 5.84 ml)].

3.4. Chocolate-flavored milk re-intake (post-extraction)

Three-way ANOVA [group x test day (A, B, C) x interval (extraction, re-intake at 20 min)] revealed that the effect of group [$F(1,20) = 11.88$; $p < 0.0025$], days [$F(2,40) = 145.97$; $p < 0.000001$], and interval [$F(1,20) = 18.31$; $p < 0.00036$] and the interaction of group x days x interval were significant [$F(2,40) = 4.91$; $p < 0.0123$].

Post-hoc analysis showed that groups significantly differed in cumulative intake at 20 min in experiment 1A [$p < 0.001$, (LPBe: 1.5 ml, Control: 3.81 ml)] and 1C [$p < 0.0003$, (LPBe: 2.58 ml, Control: 6.0 ml)] (Fig. 2) but not in experiment 1 B [$p < 1$, (LPBe: 0.45 ml, Control: 0.45 ml); (Fig. 3). In the control group, the difference between the amount of food extracted and the cumulative re-intake at 20 min was not significant in either experiment 1A ($p < 0.74$) or 1C ($p < 1$). In contrast, this difference was significant in the

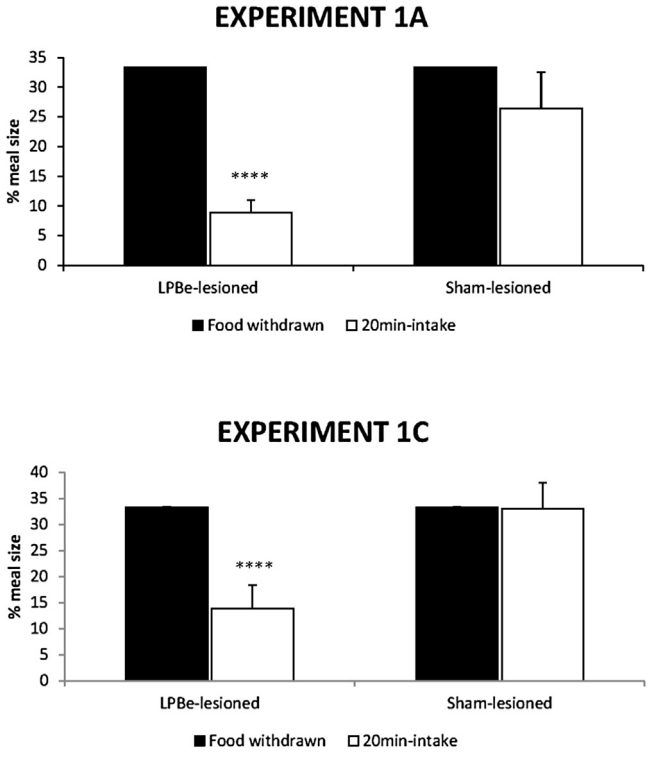

Fig. 2. Mean intake (% meal size) of liquid diet (chocolate-flavored milk) by animals in experiments 1A and 1C (LPBe-lesioned and sham-lesioned) in the re-ingestion test at 20 min (****: $p < 0.001$).

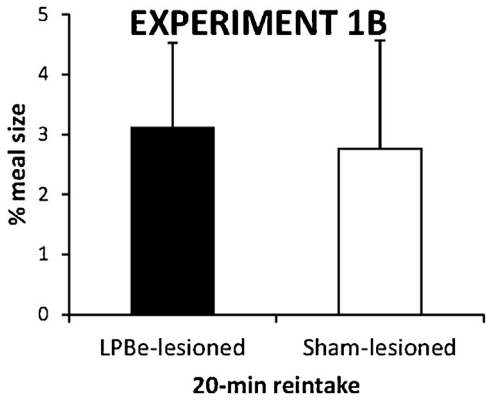

Fig. 3. Mean intake (% meal size) of liquid diet (chocolate-flavored milk) by animals in experiment 1 B (LPBe-lesioned and sham-lesioned) at 20 min.

LPBe-lesioned group in both experiments 1A ($p < 0.00013$) and 1C ($p < 0.00016$) (Fig. 2).

Post-hoc analysis showed that neither the LPBe-lesioned ($p < 0.92$) nor the sham-lesioned ($p < 0.79$) animals differed in their behavior between experiments 1A and 1C. In contrast, the re-intake volume at 20 min did not significantly differ between experiments 1A and 1 B in the LPBe-lesioned group ($p < 0.844$) but significantly differed between these experiments in the sham-lesioned group ($p < 0.00013$) (Fig. 4).

4. Discussion

Under the present experimental conditions, this study found no difference in liquid food satiation between food-deprived LPBe-lesioned and neurologically intact controls, and both groups appeared to regulate meal size in a normal manner under the present experimental conditions, i.e., in animals with previous (pre-surgery) experience of the diet. However, the intake behavior of these groups significantly differed when one-third of the stomach food content was withdrawn immediately after reaching satiation on the liquid diet. The post-extraction intake (re-intake) of the LPBe-lesioned animals failed to compensate for the deficit, with a significantly lower re-intake than the gastric amount withdrawn; unlike the control animals, whose re-intake matched the amount lost (Fig. 2) (Davis and Campbell, 1973; Deutsch et al., 1978; Kaplan et al., 1994; Snowdon, 1970; Wirth and Mchugh, 1983).

Therefore, although there did not appear to be interference with the control of the satiation process in food-experienced LPBe-lesioned animals, these lesions affected the capacity of the animals to adjust their behavior to the gastrointestinal treatment, i.e., extraction of one-third of the previously consumed liquid diet. However, when no nutrients were withdrawn after satiation (experiment 1B), the behavior of the two groups was again identical, with no difference in any study variable (Fig. 3).

Comparison of the results for LPBe-lesioned animals between experiments 1A and 1B only showed significant differences in the 20-min re-intake interval. The fact that removal of food from the stomach did not modify the behavior of these animals indicates that they may be unable to detect changes in the food present in their stomachs. In contrast, re-intake results for the neurologically intact control animals significantly differed between experiments 1A and 1B after all time intervals (Fig. 4).

In experiments 1A, 1B, and 1C, the amounts of liquid diet consumed and the time to reach satiation (pre-food extraction) were not very high in either group. It could be argued that the period of 5 min without intake was too brief to ensure that full satiation had been reached, which might therefore have influenced the post-extraction food intake. However, in experiment 1B, in which no food was extracted, the initial food intake and the intake duration of both LBPe-lesioned and control rats were similar to the values recorded in experiment 1A, and neither group in this experiment showed an appreciable consumption of food for at least 25 min after the initial intake, indicating that satiation had been fully reached.

The effect of aspirating one-third of the gastric cavity content appeared to be lesser in the second test (experiment 1C), but neither LPBe-lesioned animals nor the sham-lesioned differed in their behavior between experiments 1A and 1C. In contrast, a certain adaptation can be observed in the larger amounts of the diet consumed both pre-extraction (LPBe-lesioned, 16.36 vs. 19.2; Sham-lesioned 14.81 vs. 17.62) and in the re-intake at 20 min post-extraction (LPBe-lesioned, 1.5 vs. 2.58; Sham-lesioned 3.81 vs. 6.0).

It could be suggested that the decreased liquid food re-intake in LPBe animals (experiment 1B) might be attributable to secondary effects induced by the experimental conditions (malaise, weakness, locomotion, etc.) rather than to the interruption of information from the gastrointestinal tract. However, we consider this to be an unlikely possibility because their body weight always remained slightly higher than that of the control animals, although the difference was not statistically significant. In fact, LPB lesions have been found to produce a long-term increase in food intake and body weight (Agüera et al., submitted, Nagai et al., 1987), while it has also been observed that LPBe lesions do not interfere with the establishment of flavor preferences in sequential learning tasks (Zafra et al., 2002). In addition, conditioning taste aversion studies have demonstrated that a single learning trial is sufficient to induce aversion, unlike in our experiment 1C. Finally, if the LPBe lesion had induced malaise or weakness, these effects would also have been observed at any of the times when the liquid (pre-training test, satiation test) or solid diet was presented, which was not the case.

We did not determine the origin of specific visceral signals that are blocked by the LPBe and may be responsible for the effects observed in experiments 1A and 1C. However, LPBe lesions may have interrupted the processing of gastric and intestinal signals in these animals, because extraction would have reduced the amount of food in both segments. In fact, information is transmitted to the brain through the vagus nerve from both levels of the digestive system (Altschuler et al., 1989; Sengupta and Gebhart, 1994; Shapiro and Miselis, 1985), and information is received in this parabrachial subnucleus from both gastrointestinal segments (Baird et al., 2001; Kobashi et al., 1993; Suemori et al., 1994; Yamamoto and Sawa, 2000a, 2000b; Wang et al., 1992).

The vagus nerve processes not only volumetric/mechanical but also chemical gastrointestinal information (Ladenheim and Ritter, 1991; Mei, 1983; Phillips and Powley, 1998; Sengupta and Gebhart, 1994; Smith et al., 1981; Uneyama et al., 2004; Yamamoto and Sawa, 2000a), and both types of data appear to be processed through the LPBe, whose neurons were found to be sensitive to stomach distension (Baird et al., 2001; Suemori et al., 1994) as well as to the intragastric (Yamamoto and Sawa, 2000a,b), intestinal (Wang et al., 1992) presence of nutrients or food-related chemical signals (Li and Rowland, 1995; Rowland et al., 1997; Smith et al., 1981; Swick et al., 2015; Yang et al., 2004).

The present results are consistent with previous reports that the LPBe may be part of the visceral vagal-NSTic axis involved in processing information from the upper gastrointestinal tract and, therefore, in food intake regulation (Becskei et al., 2007; Horn and Friedman, 1998a, 1998b; Li and Rowland, 1995, 1996). According to our findings, this pathway would be particularly important in circumstances requiring a rapid detection and processing of visceral information. LPBe lesions do not appear to affect the control of satiation when previously known foods are offered (the volume of the initial intake was identical in both groups), but the importance of the pontine LPBe subnucleus becomes evident when there

Fig. 4. Mean intake (% meal size) of liquid diet (chocolate-flavored milk) by LPBe-lesioned and sham-lesioned animals in experiments 1A (with food withdrawal) and 1 B (without food withdrawal) in the re-ingestion test at 20 min. (***: $p < 0.01$).

is a need to rapidly detect and immediately compensate for a deficit (extraction of 1/3 of the material ingested at 5 min after satiation).

Studies of taste discrimination learning also found that an intact LPBe (and NSTc or vagus nerve) is essential in tasks that require the rapid processing of intragastrically administered substances associated with one of the taste stimuli. However, these structures do not appear to be important in interoceptive sequential learning tasks, in which rapid visceral processing is not an essential requirement (Mediavilla et al., 2000, 2011; Zafra et al., 2002, 2006, 2007).

Participation of the vagus nerve in satiation is well-documented (Gonzalez and Deutsch, 1981; Mordes et al., 1979; Phillips and Powley, 1998; Smith et al., 1981; Snowdon, 1970), but various studies have demonstrated that this behavior is only transiently affected by the ablation of vagal afferents. Thus, although a significant increase in meal size is observed immediately after surgery, the effect is compensated for and disappears in subsequent sessions (Phillips and Powley, 1998; Zafra et al., 2003). Hence, although the satiation might be controlled through the vagal axis under normal conditions, alternative routes (humoral, splanchnic) may become involved when this pathway is interrupted, compensating for the lack of information *via* the vagal axis (Deutsch and Jang Ahn, 1986; Furness et al., 2001; Hamr et al., 2015). Likewise, transient overintake was observed after ablation of vagal afferents [and of the LPBe (Agüera, 2012; Agüera et al., submitted)],when a novel diet was offered and there was no previous experience to guide intake behavior, and the volume of food intake only normalized after successive presentations (Chavez et al., 1997).

However, this compensatory mechanism does not operate in a re-intake test requiring rapid sensory information, and this type of task may depend on the integrity of the above-mentioned neuroanatomical axis. Thus, despite the experience of an identical experiment two days earlier (experiment 1A), the present LPBe-lesioned animals were not able to compensate for the deficit created by partial extraction of their gastric contents (experiment 1C).

We have no data available to determine whether the intake of lesioned animals is finally the same as that of controls, although it is well known that information from the gastrointestinal tract can reach the brain *via* different complementary pathways (vagal, spinal, humoral), which, alongside the participation of learning processes, means that a long-term compensatory effect can be expected.

The protocol adopted in the present study (extraction of one-third of the food in the gastric cavity and analysis of subsequent re-intake) may possibly be analogous, in our view, to certain clinical conditions such as bulimia nervosa, which is characterized by the voluntary induction of vomiting (partial withdrawal of gastric content) and subsequent food intake (Kaye, 2008). Indeed, structures that form part of this visceral-brain pathway (vagus nerve) have been implicated in this disorder (Faris et al., 2016).

Finally, we highlight that, in parallel with the regulatory processes described in the present paper for controlling the volume of food intake, the LPB area is a brainstem region that participates in both aversive (Agüero, 1990; Carter et al., 2013, 2015; Garfield et al., 2015; Mediavilla et al., 2000; Rinaman and Dzmura, 2007; Zafra et al., 2005) and rewarding (Zafra et al., 2002) nutritional processes.

In summary, these findings suggest that the LPBe is a brain region involved in food intake behaviors, as in the case of a rapid re-intake task, in which the deficits induced cannot be compensated for in the absence of this pontine subnucleus. The normal satiation process offers an initially comparable example, given that interruption of the vagal-LPBe axis (Agüera, 2012; Agüera et al., submitted; Zafra et al., 2003) induces a short-term increase in the intake volume, although this disorder is partially compensated for, presumably through experience or long-term post-absorptive mechanisms.

In conclusion, the LPBe, as part of a neuroanatomical axis that appears to include the vagus nerve and the NSTic, may be particularly decisive in food intake processes when rapid detection of gastrointestinal information is essential and cannot be compensated for by alternative systems.

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