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Increased short-term food intake after external lateral parabrachial subnucleus lesions in rats

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The vagus nerve and several brainstem nuclei to which it projects have been closely associated with food intake. The aim of this study was to determine the degree to which the same or different information on food intake is processed by this nerve and by one of these nuclei, the external lateral parabrachial subnucleus (LPbNe). For this purpose, we analyzed the solid and liquid food intake of Wistar rats subjected to vagal deafferentation with capsaicin or lesions of the LPbNe. Vagotomized animals consumed significantly larger amounts of solid food during the first 24 h post-surgery but not at 48, 72, or 96 h. Animals with LPbNe lesions also consumed larger amounts of liquid and solid foods but only during periods of 60 min on day 5 and 90 min on day 6 post-surgery, respectively. According to these findings, both the vagus nerve and the LPbNe appear to be involved in short-term regulation of food intake, although they participate over different time scales. These data are discussed in terms of the potential importance of the vagal-parabrachial axis in the rapid processing of nutritional information from the upper gastrointestinal tract.

Key words: vagus nerve afferents, capsaicin, external lateral parabrachial subnucleus, short-term food intake, Wistar rats

INTRODUCTION

Short-term satiety (or satiation) is a motivational behavior that appears to largely depend on visceral-cerebral neurobiological mechanisms (Snowdon and Epstein, 1970; Deutsch, 1990; Kaplan et al., 1993; Schwartz et al., 1999; Hamr et al., 2015). In normal conditions, information relating to food originating from different levels of the gastrointestinal tract is transmitted *via* neural pathways to the central nervous system, where they appear to decisively contribute to cessation of food intake (Molina and Puerto, 1986; Schwartz, 2000). The peripheral neural mechanism responsible for this process appears to particularly involve the sensory component of the vagus nerve (Mordes et al., 1979; González and Deutsch, 1981; Smith et al., 1981; Altschuler et al., 1989; Sengupta and Gebhart, 1994; Phillips and Powley, 1998; Schwartz et al., 1999; Schwartz, 2000; Zafra et al., 2003; Berthoud, 2008; Czaja et al., 2008; Peters et al., 2013), a cranial nerve whose afferents are widely distributed throughout the digestive system (Loewy, 1990; Prechtl and Powley, 1990).

It has been verified that complete resection of the vagus nerve (Phillips and Powley, 1998) and vagal deafferentation by treatment with capsaicin (Castonguay and Bellinger, 1987; Chavez et al., 1997; Kelly et al., 1999; Zafra et al., 2003; 2004), a neurotoxin which transiently and selectively destroys weakly myelinated A-delta or unmyelinated C afferent fibers (Hölzer, 1991; Ritter and Dinh, 1992; Czaja et al., 2008; Gallaher et al., 2011), both produce an initial increase in food consumption that is subsequently counteracted and normalized. These results have been interpreted by multiple authors as the consequence of a transient disorder in processes that regulate short-term satiety. It appears that systems other than the affected vagal fibers subsequently com-

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pensate for the deficits generated by the lesion (Kelly et al., 2000; Zafra et al., 2003).

The vagal afferents responsible for processing visceral signals from different gastrointestinal tract segments project towards various subnuclei located in the intermediate-caudal area of the nucleus of the solitary tract (NTSic) (Shapiro and Miselis, 1985; Norgren and Smith, 1988; Altschuler et al., 1989). The NTSic is a brain gateway for visceral signal processing (Roman et al., 2016) in which information is organized in a viscerotopic manner. In this way, information from the stomach is usually sent to the dorsomedial subnucleus of the NTSic, whereas information from the small intestine is distributed in more caudal and medial areas (Altschuler et al., 1989). In turn, these subnuclei transmit visceral information from the gut to several parts of the lateral division of the pontine parabrachial complex (Loewy and Burton, 1978), including the external lateral parabrachial subnucleus (LPbNe) (Herbert et al., 1990; Loewy, 1990; Bernard et al., 1993; Saper, 1995), which that may be part of the anatomical axis through which the vagus nerve participates in short-term nutrition.

In agreement with this hypothesis, various researchers have observed hyperphagic behaviors after large lesions of the parabrachial complex (Yamamoto et al., 1995) and also after lesions affecting the entire lateral parabrachial area (LPbN) (Nagai et al., 1987; Takaki et al., 1990; Zafra et al., 2005). These lesions appear to include the LPbNe, a subnucleus that has generally been related to the processing of visceral-sensory information (Herbert et al., 1990; Moga et al., 1990; Bernard et al., 1993; De Gobbi et al., 2001; Tanaka et al., 2004; Hurtado et al., 2014, 2017; Zafra et al., 2016).

Large lesions of the LPbN also block the effects of certain pharmacological or endocrine agents on food intake (Calingasan and Ritter, 1993; Trifunovic and Reilly, 2001; Becskei et al., 2007). Both the LPbN activation induced by these agents and the consequent food intake modifications can be prevented or attenuated by vagotomy (Smith et al., 1981; Ritter et al., 1994; Li and Rowland 1995; Horn et al., 2001; Yang et al., 2004; Abbott et al., 2005). Thus, the LPbNe subnucleus appears to be one of the various areas at which these substances act (Li and Rowland, 1994; 1995; Li et al., 1994; Ritter et al., 1994; Elmquist et al., 1997, 1998; Rowland et al., 2000; Trifunovic and Reilly 2001). It has also been confirmed that neurons of the LPbN subnucleus, which apparently includes the LPbNe, can be activated by visceral interventions such as gastric distension (Suemori et al., 1994; Baird et al., 2001a; b). This information appears to be processed and sent to the brain *via* the vagal pathway (Mei, 1983; Cervero, 1994; Sengupta and Gebhart, 1994) and has frequently been related to the regulation of short-term food intake (Deutsch, 1985; Phillips and Powley, 1996, 1998; Powley and Phillips, 2004; Berthoud 2008).

Previously published results indicate that animals with vagal deafferentation would have larger initial intakes on their first exposures to food because they lack the necessary vagal visceral-sensory fibers responsible for short-term satiety processes. Later, however, there may be a subsequent compensatory effect if food remains available *via* complementary regulatory mechanisms that remain intact or even *via* vagal pathway fibers resistant to capsaicin treatment (Deutsch and Jang Ahn, 1986; Furness et al., 2001; Zafra et al., 2003; Hamr et al., 2015).

Considering this background, and given the connections between the vagus nerve and LPbNe, the objective of this study was to examine and compare the short-term solid and liquid food intake of animals subjected to either vagal deafferentation induced by local treatment with capsaicin or lesioning of the LPbNe subnucleus.

The study hypothesis was that if these two structures participate in the same pathway for processing nutritional information of gastrointestinal origin, animals with lesions of the LPbNe, one of the brainstem nuclei that processes information from vagal visceral-sensory afferents (Loewy, 1990; Bernard et al., 1993; Saper, 1995), may exhibit intake behavior analogous to that of vagotomized animals (Castonguay and Bellinger, 1987; Chavez et al., 1997; Phillips and Powley 1998; Kelly et al., 1999; Zafra et al., 2003; 2004), with a short-term but not long-term increase in solid and liquid food intake.

METHODS

Subjects

This study consists of two experiments that used 42 Wistar rats (weighing 279-302 g at baseline) randomly distributed into four groups: capsaicin-treated group (n=12) and its control group (n=10), and LPbNe-lesioned group (n=10) and its sham lesion control group (n=10).

Animals were individually housed in methacrylate cages (30x15x30 cm) in which the experiments were also carried out. The laboratory was maintained at 22-24°C with a 12:12 light/dark cycle (lights on at 8 am). Experiments were performed during light periods with white noise. Food and water were available to the animals *ad libitum*.

All surgical techniques and behavioral procedures complied with Spanish legislation [Royal Law

(1201/2005)] and the European Community Council Directive (86/609/EEC).

Surgical procedure: Vagal deafferentation

Vagal deafferentation was achieved by using capsaicin, one of the most common surgical methods for this purpose (Jancsó et al., 1987; Raybould and Taché, 1989; Hölzer, 1991; Berthoud and Neuhuber, 2000; Blackshaw et al., 2000), following the procedure of Raybould and Taché (1989). Animals were anesthetized with sodium pentothal (56.3 mg/kg i.p. Sodium Thiopental, Abbot Laboratories, Abbot Park, IL, USA), and an incision of approximately 3 cm was made in the midline of the abdominal wall. After exteriorizing the stomach and esophagus, a paraffin lamina was placed under the esophagus to avoid capsaicin (Fluka, 98%) propagation to surrounding tissues. The esophagus was then surrounded with cotton impregnated with the capsaicin solution [1 mg capsaicin dissolved in 1 mL vehicle (10% Tween 80 in olive oil)]. The cotton was soaked every 5 min, applying a total of 1 mL capsaicin/animal. After the 30-min application of capsaicin, the area was washed with saline solution and dried with sterile material. The incision was closed with several sutures, and topical antiseptic (Betadine, Sarget Lab, Merignac, France) was applied on the wound, followed by intramuscular administration of 0.1 mL penicillin (1.000.000 IU, Penilevel, Lab. Ern, Barcelona, Spain) as a prophylactic measure. The control group underwent an identical surgical procedure except for the perivagal administration of vehicle alone (10% Tween 80 in olive oil), without the application of capsaicin.

Behavioral procedure

The same behavioral procedure was followed in both experiments (Fig. 1). The amount of solid food consumed during the 24 h before surgery was considered to be the baseline solid food intake. The animals were returned to their cages immediately after surgery with water and solid food *ad libitum* (Alimento de Laboratorio. Dietas Panlab. Panlab S.L., Barcelona, Spain), and their intake was recorded after 24, 48, 72, and 96 h post-surgery. For this purpose, the animals were offered a stock diet at the corresponding hour each day, and the amount that remained 24 h later was withdrawn and quantified to the nearest 0.1 g.

On day 4 post-surgery, after the last solid food recording (96 h), animals were deprived of water and food 24 h later, on day 5 post-surgery, they were offered a sucrose solution (10% diluted in water) through two graduated burettes, and their intake was recorded to the nearest 0.1 cc at 15, 30, and 60 min. After the last measurement (60 min), the sucrose solution remained available to the animals until the next day (day 6), when their consumption was again quantified (sucrose solution intake 24 h after being offered). On the same day (day 6), after withdrawing and quantifying the sucrose, solid food was again offered *ad libitum* to the animals, recording their intake to the nearest 0.1 g after 30, 60, and 90 min.

Two animals in the LPbNe group died during the 7-day post-surgery recovery period, therefore, this group ultimately comprised eight animals.

The body weight of the animals was recorded daily during the experimental period (see Fig. 1).

Vagotomy test

After vagal deafferentation, the animals underwent the vagotomy test proposed by Martin et al. (1978) to establish whether the vagus nerve had accidentally suffered total section during surgery (afferent and efferent fibers), which would exclude the animal from the data analyses. The test involved the extraction and weighing the stomach of animals after 12 h of fasting, and a complete vagotomy was defined when the ratio of stomach weight to pre-fasting animal weight was >0.02.

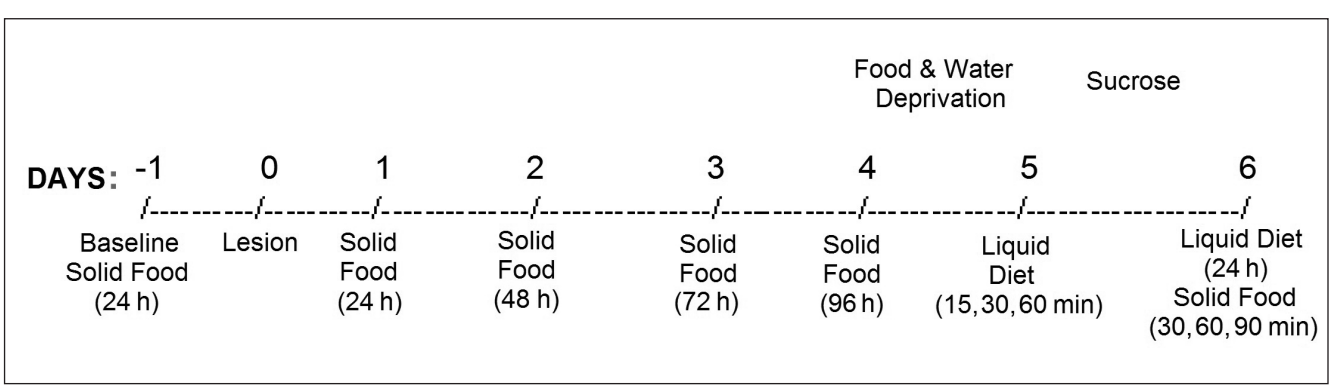

Fig. 1. Timeline of intake measurements in this study.

Surgical procedure: Lesion of external lateral parabrachial subnucleus (LPbNe)

For the bilateral electrolytic LPbNe lesions, animals received general anesthesia with sodium pentothal (50 mg/kg i.p. Sodium Thiopental, Abbot Laboratories). A stereotaxic device (model SAS-4100, Bilaney, Dusseldorf, Germany) was utilized [Coordinates: AP=-0.16 mm, L=+-2.4 mm, V=+3.0 mm, Interaural=-0.16 mm, according to the neuroanatomical atlas of Paxinos and Watson (1998)], and the animals received a cathodic electric current (0.3 mA) for 10 s from a DCLM-5 lesion generator (Grass Instruments, Quincy, MA, USA) through a monopolar 00 stainless steel electrode insulated except at the tip.

In the sham lesion control group, all of the above steps were followed except that the vertical coordinate was +3.5 mm (to avoid affecting the LPbNe) and no current was applied.

Histology

Animals in the LPbNe group were anesthetized with a sodium pentothal overdose (80 mg/kg ip) and intracardially perfused with 10% formaldehyde. Brains were removed and stored in 10% formaldehyde before lamination.

Electrolytic lesions were localized, and their extension was measured on photographs (VMZ-4F stereoscopic magnifying glass and PM-6 camera, Olympus, Tokyo, Japan) of Cressyl Violet-stained 40-micron coronal sections (1320M microtome-freezer, Leitz, Wetzlar, Germany) (see Fig. 2).

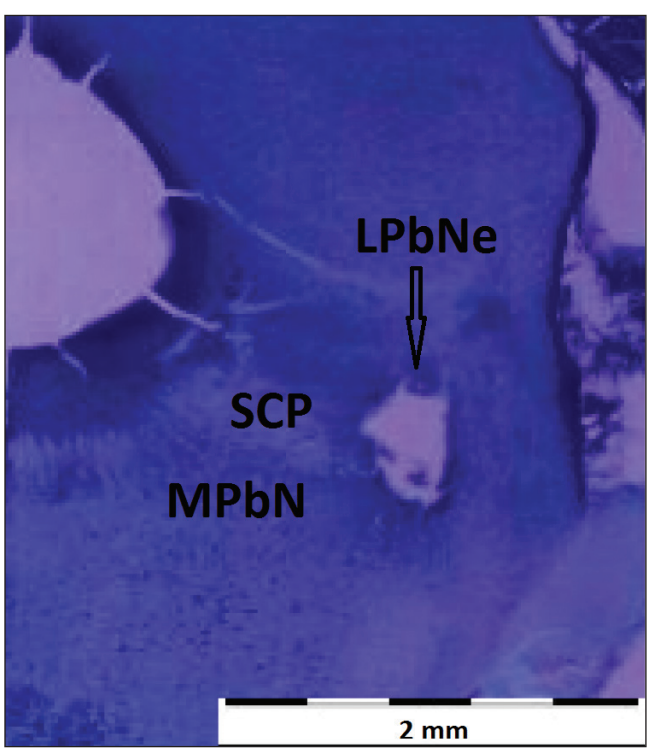

Fig. 2. Anatomical localization of the LPbNe electrolytic lesion of the right hemisphere [Interaural = -0.16 mm according to the neuroanatomical atlas of Paxinos and Watson (1998)]. Scale bar is 2 mm. LPbNe: external lateral parabrachial subnucleus, MPbN: medial parabrachial nucleus, SCP: superior cerebellar peduncle.

Statistical analysis

STATISTICA version 6.0 (Statsoft Inc, OK) was used for statistical analyses. One-way ANOVA was used to analyze the results of the vagotomy tests. The mean body weight in both groups before surgery (baseline) and at 24 h were analyzed using ANOVA (group x weight), and the mean solid food and sucrose solution intakes on all days were analyzed using a two-way repeated-measures ANOVA (group x session). Values were expressed as means ± SEM. Significant effects were evaluated with the *post hoc* TUKEY HSD test, and $p<0.05$ was considered significant.

RESULTS

Vagotomy test

According to the test results, no animal suffered complete vagotomy, and no statistically significant differences were observed between the capsaicin-treated and control groups in the ratio between stomach weight and total pre-fasting body weight ($F_{1,20}$=0.09,

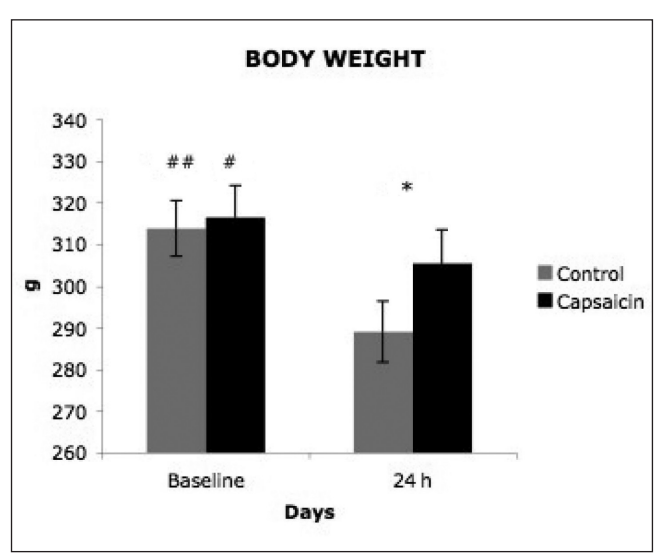

Fig. 3. Body weight of capsaicin-treated (Capsaicin) and Control groups the day before surgery (baseline) and 24 h post-surgery. * p<0.001 Control *vs.* Capsaicin, # p<0.01, ## p<0.001 BL *vs.* 24 h.

P<0.76). Accordingly, the data for all animals were included in the statistical analyses.

Pre-surgical data (baseline of solid food intake and body weight)

The capsaicin-treated group and its control group did not significantly differ in mean daily solid food intake during the 24 h before surgery ($F_{1,20}$=0.25, P<0.61) or in mean body weight ($F_{1,20}$=0.06, P<0.80).

The LPbNe-lesioned group and its control group did not significantly differ in their daily solid food intake during the 24 h before surgery ($F_{1,16}$=0.03, P<0.84) or in their body weight on the day before surgery ($F_{1,16}$=0.15, P<0.69).

Post-surgical data

Body weight (baseline vs. 24 h post-surgery)

The comparison of body weight of the capsaicin-treated group and its control group between the day before surgery (baseline) and at 24 h post-surgery showed that the group effect was not significant ($F_{1,20}$=0.81, P<0.37) but the session effect ($F_{1,20}$=69.38, P<0.001) and interaction ($F_{1,20}$=10.04, P<0.004) were. *Post-hoc* analysis showed a significant body weight reduction between these time points in both groups (capsaicin group: P<0.005, control group: P<0.001) and a significant difference between the groups at 24 h post-surgery (P<0.001) (see Fig. 3).

Analysis of body weight of the LPbNe-lesioned group and its control group between the day before surgery (baseline) and 24 h post-surgery showed that the session effect was significant ($F_{1,16}$=68.62, P<0.001) but the group effect and the interaction were not (P>0.05), and no significant between-group difference in body weight reduction was observed on either day.

Solid food intake (24, 48, 72, and 96 h post-surgery)

Analysis of the solid food intake of the capsaicin-treated group and its control group found that the group effect was not significant ($F_{1,20}$=3.65, P<0.07) but the session effect ($F_{3,60}$=88.49, P<0.001) and the interaction ($F_{3,60}$=6.89, P<0.001) were. *Post-hoc* analysis showed a higher intake in the capsaicin-treated *versus* control group during the 24 h after surgery (P<0.001), with no significant difference between them at 48, 72, and 96 h post-surgery (all P>0.05) (see Fig. 4).

Post-hoc analysis demonstrated a significant intake reduction in both groups between the day before surgery (baseline) and 24 h post-surgery (control group: 84.33%, capsaicin-treated group: 55.32%) (all P<0.001), with a significantly greater reduction in the controls (P<0.001).

Analysis of solid food intake of the LPbNe-lesioned group and its control group showed that the session effect was significant ($F_{3,48}$=16.66, P<0.001) but the group effect ($F_{1,16}$=0.14, P<0.70) and the interaction ($F_{3,48}$=0.026,

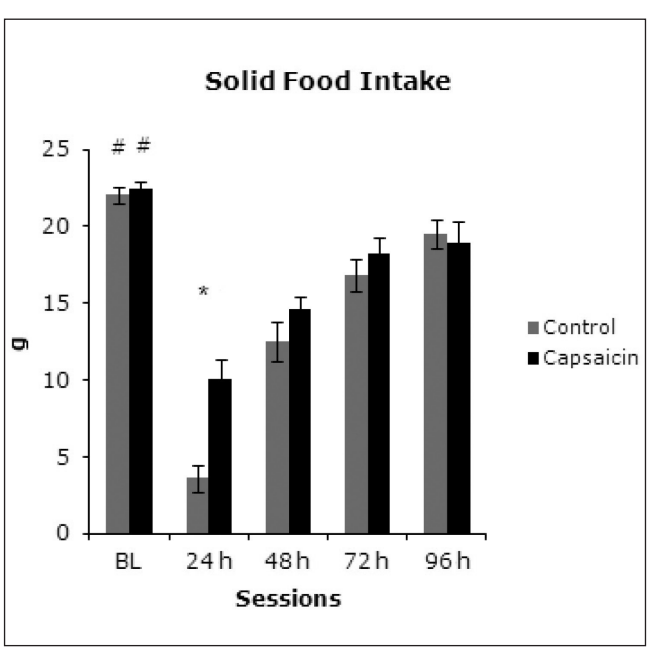

Fig. 4. Solid food intake of capsaicin-treated (Capsaicin) and Control groups in sessions of 24, 48, 72, and 96 h post-surgery. BL: Baseline solid food intake. * p<0.001 Control *vs.* Capsaicin, # p<0.001 BL *vs.* 24 h.

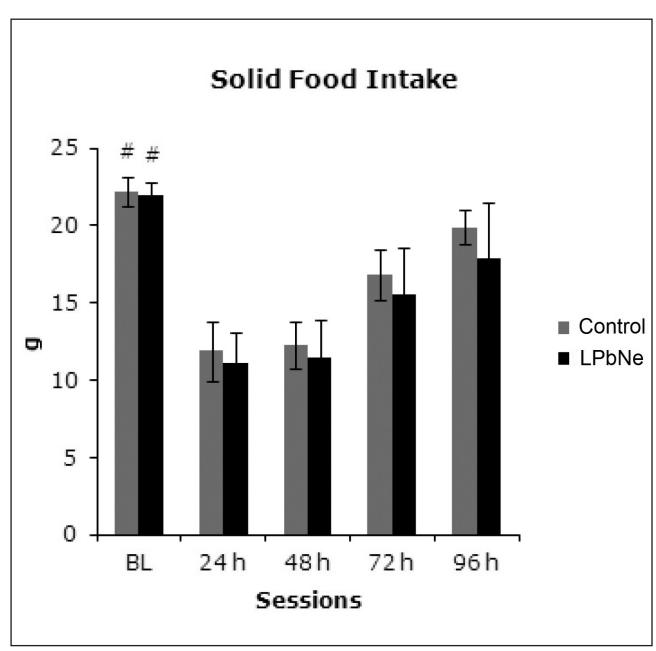

Fig. 5. Solid food intake of the LPbNe and Control groups in sessions of 24, 48, 72, and 96 h post-surgery. BL: Baseline solid food intake. # p<0.001 BL *vs.* 24 h.

P<0.99) were not, and no differences between groups were observed in any session (see Fig. 5).

Post-hoc analysis showed a significant intake reduction in both groups between the day before surgery (baseline) and 24 h post-surgery (control group: 47.01%, LPbNe group: 50.88%) (all *P*<0.001), with no significant difference in this reduction between the groups (*P*<0.75).

Sucrose solution intake (Day 5: 15, 30, and 60 min)

Analysis of the sucrose solution intake of the capsaicin-treated group and its control group showed that the session effect ($F_{2,40}$=54.52, *P*<0.001) was significant but the group effect ($F_{1,20}$=0.074, *P*<0.78) and the interaction ($F_{2,40}$=0.075, *P*<0.92) were not, i.e., there were no differences between the groups in any session.

Analysis of the sucrose solution intake of the LPbNe-lesioned group and its control group showed that the group effect was not significant ($F_{1,16}$=1.12, *P*<0.30) but the session effect ($F_{2,32}$=11.30, *P*<0.001) and the interaction ($F_{2,32}$=6.33, *P*<0.004) were. *Post-hoc* analysis revealed a higher sucrose solution intake by animals with LPbNe lesions in a 60-min test after 24 h of water and food deprivation (*P*<0.005), but not at 15 or 30 min (all *P*>0.05) after the presentation of food and water (see Fig. 6).

Sucrose solution intake (Day 6: 24 h)

No significant differences in sucrose solution intake of the capsaicin-treated ($F_{1,20}$=0.12, *P*<0.72) and LPbNe-lesioned ($F_{1,16}$=0.001, *P*<0.97) groups with their respective control groups on day 6 (24 h period).

Solid food intake (Day 6: 30, 60, and 90 min)

Analysis of solid food intake of the capsaicin-treated group and its control group showed that the session effect ($F_{2,40}$=46.49, *P*<0.001) was significant but the group effect ($F_{1,20}$=1.34, *P*<0.25) and the interaction ($F_{2,40}$=0.65, *P*<0.52) were not, with no differences between groups in any session.

Analysis of solid food intake of the LPbNe-lesioned group and its control group showed that the group effect ($F_{1,16}$=7.80, *P*<0.013), session effect ($F_{2,32}$=19.09, *P*<0.001), and their interaction ($F_{2,32}$=3.76, *P*<0.034) were all significant. *Post-hoc* analysis of solid food intake between groups in each individual session showed that, although there were no differences at 30 (*P*<0.38) and 60 min (*P*<0.14) after solid food presentation, a larger amount was consumed by the LPbNe group than by the control group in the 90-min test (*P*<0.001) (see Fig. 7).

DISCUSSION

This study demonstrates that an initial increase in food intake is induced in rats both by vagus nerve deafferentation with perivagal capsaicin administration and by lesioning of the LPbNe subnucleus, one of the projection centers of the vagus in the brainstem.

The results confirm previous findings by our group that capsaicin-treated animals consume larger amounts

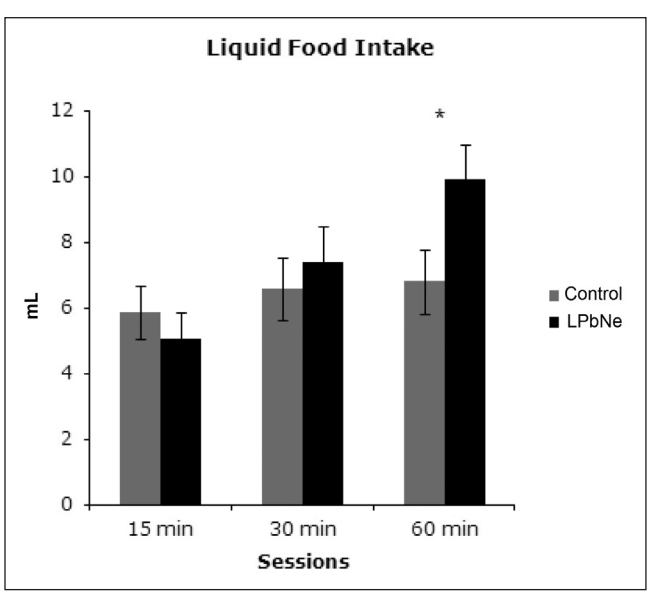

Fig. 6. Sucrose solution intake of the LPbNe and Control groups in sessions of 15, 30, and 60 min of day 5. * p<0.01 Control *vs.* LPbNe.

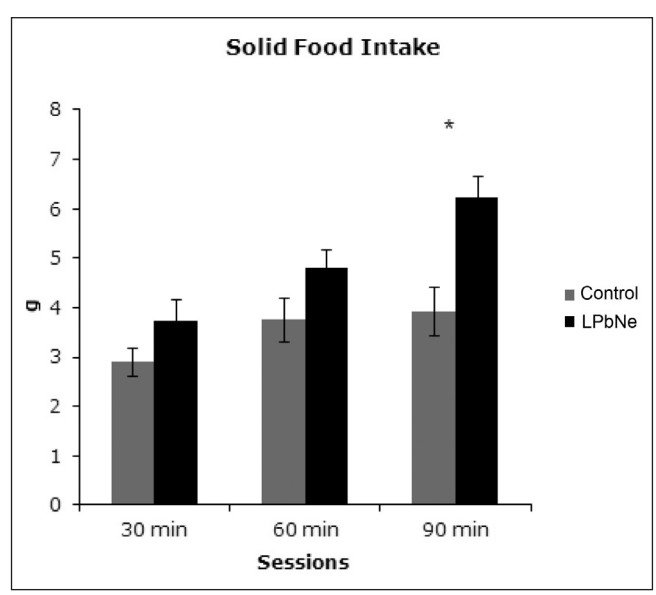

Fig. 7. Solid food intake of the LPbNe and Control group in sessions of 30, 60, and 90 min on day 6. * p<0.001 Control *vs.* LPbNe.

of solid food than their controls during the first 24 h post-surgery but not after 48, 72, or 96 h (Zafra et al., 2003, 2004, 2007, 2017a). Other authors have also demonstrated a transient food intake increase in animals treated with capsaicin (including when i.p. administered). This effect is particularly marked when the solid or liquid food offered is not familiar to the animals (Chavez et al., 1997; Kelly et al., 1999, 2000) or when complete diets are offered (Zafra et al., 2003, 2017a).

Capsaicin is a neurotoxin whose perivagal administration selectively affects visceral-sensory afferents (Hölzer, 1991; Ritter and Dinh, 1992), which are important in processes related to short-term satiety (Snowdon and Epstein, 1970; Mordes et al., 1979; González and Deutsch, 1981; Deutsch, 1990; Phillips and Powley, 1998; Zafra et al., 2003, 2004).

However, in contrast to these studies, our experiment showed no differences between the capsaicin-treated and control groups in the intake of non-familiar sucrose solution offered on day 5 post-surgery. Besides the utilization of different capsaicin administration pathways (perivagal in the present study), this discrepancy may be attributable to other factors, such as (1) our use of a 60-min test for the first exposure to the non-familiar sucrose solution in comparison to the test duration of up to 180 min in the above studies, or (2) the nature of the food (carbohydrates *vs.* fats) and its complexity (complete/fats *vs.* disaccharide).

The results showed that larger amounts of sucrose solution and solid food were consumed by LPbNe-lesioned animals than by controls at 60 min and 90 min (see Figs 6 and 7, respectively) but that their intakes were similar after longer periods (≥24 h) (see Fig. 5 and day 6 of experiment 2). These findings are consistent with the hyperphagic behavior observed after lesions of the whole parabrachial complex (Yamamoto et al., 1995) or large lesions of the LPbN area (Nagai et al., 1987; Takaki et al., 1990; Zafra et al., 2005). Thus, more specific lesions of one of the subnuclei (LPbNe) mean that the effect described can be reproduced, allowing a more precise anatomical localization of the parabrachial substrate involved in short-term food intake. Our results are also in line with recent reports that meal size is increased by the inhibition of calcitonin gene-related peptide-expressing neurons in the outer external lateral subdivision of the parabrachial nucleus (Carter et al., 2013; Campos et al., 2018).

Given that most of the food appears to remain in the gastrointestinal tract at the end of each intake session (McHugh et al., 1975), it has been proposed that food intake cessation would depend on the rapid processing of gastrointestinal sensory signals (Davis and Campbell, 1973; McHugh et al., 1975; Kraly and Smith, 1978; Wirth and McHugh, 1983), in which the vagus nerve appears to participate (Phillips and Powley, 1998; Zafra et al., 2003, 2006, 2007, 2017a). In this regard, various studies have proposed that gastric distension, which is mainly transmitted by the vagal pathway (Mei 1983; Cervero, 1994; Sengupta and Gebhart, 1994), participates in satiation control (Deutsch, 1985; Phillips and Powley, 1996, 1998; Powley and Phillips, 2004; Berthoud, 2008). In fact, it has been observed that neurons of the LPbN nucleus, which apparently includes the LPbNe subnucleus, are especially sensitive to gastric distension (Suemori et al., 1994; Baird et al., 2001a, b). Consequently, the interruption of these or other signals by the lesion may have caused the increased solid and liquid food intake observed in our study.

The LPbNe is involved in processing information on taste (Yamamoto et al., 1994; Di Lorenzo et al., 2009), and it is possible to imagine that our results are attributable to the lesion-induced blocking of this information; however, this seems to be unlikely. First, because the taste information processed by this subnucleus appears to be negative in character (HCl, quinine) and to involve more areas of the subnucleus that are more caudal than the rostral region lesioned in the present study. Indeed, when sucrose is used as a taste stimulus, the central lateral and central medial subnuclei are the most intensely activated and there is virtually no activation of the external subnucleus (Yamamoto et al., 1994). Furthermore, if lesioning of the LPbNe had affected the processing of hedonically positive taste information, our animals would have consumed a smaller amount of sucrose solution, which was not the case. It is more likely that the lesion eliminated visceral signals of satiation, given that the rostral region of the LPbNe subnucleus appears to be more involved in processing information of gastrointestinal origin (Yamamoto et al., 1994).

Taken together, the results of both experiments indicate that animals with lesions in the vagal-parabrachial axis do not adequately regulate the satiation process (Phillips and Powley, 1998; Zafra et al., 2003, 2004, 2016, 2017a). This is presumably due to the absence of the necessary afferent vagal signals or the impairment of more rostral components of this processing pathway.

However, this disruption is subsequently compensated for in both cases, either by a conditioned satiety mechanism (Treit and Spetch, 1986) or by the participation of alternative biological mechanisms that are initially non-essential in the short-term control of food intake. Thus, these mechanisms may include vagal pathways that are resistant to the action of capsaicin (Berthoud et al., 1997; Berthoud and Neuhuber, 2000). On the other hand, given that this compensatory effect was also observed to a certain extent in complete-

ly vagotomized animals (Phillips and Powley, 1998), it may be produced by the action of systems that are independent of vagus nerve action, e.g., the humoral system or splanchnic nerves of the sympathetic system (Deutsch and Jang Ahn, 1986; Furness et al., 2001; Chavez et al., 1997; Kelly et al., 1999; Zafra et al., 2003; Hamr et al., 2015).

It could also be argued that the transient nature of the effect on solid food intake and the lack of effect on the intake of sucrose solution (10%) in the capsaicin-treated animals may result from a transient neurodegenerative effect of capsaicin. In fact, recent studies reported the regeneration of vagal fibers in both capsaicin-treated and vagotomized animals (Czaja et al., 2008; Gallaher et al., 2011; Peters et al., 2013). However, this interpretation appears unlikely because the regeneration of vagal afferents is observed from 30 days post-surgery onwards, much later than the 7-day period covered by the present study.

Likewise, it is possible that brain centers other than the LPbNe may be involved in counteracting the effect induced by the lesion. In theory, these alternative nuclei could receive information from vagal and non-vagal pathways (visceral spinal nerves of the sympathetic and/or humoral system). However, according to the present results, it appears unlikely that these alternative nuclei participate in initial short-term food intake. If this were the case, the LPbNe-lesioned animals would not have shown the intake increase observed in the 60- and 90-min tests, which provide sufficient time for activation of the brain structures involved.

Our experimental data suggest that the vagus nerve and LPbNe are two components of a satiation control system, an idea compatible with neuroanatomical and neurophysiological studies (Loewy and Burton, 1978; Altschuler et al., 1989; Herbert et al., 1990). However, there was no precise time correspondence in the effect observed after interrupting these two components. Thus, no differences were observed between the capsaicin-treated and control groups during the early intake periods analyzed (15, 30, 60 min of sucrose solution intake on day 5, and 30, 60, and 90 min of solid food intake on day 6). However, the intakes of the parabrachial animals were increased during 60 min of sucrose solution intake on day 5 and during 90 min of solid food intake on day 6. We consider that the absence of an early effect in the capsaicin-treated group may be related to the unpleasant effects of the noxious surgery itself, given that the vagal action of capsaicin does not appear to block either nociceptive processes or the anorexic effect induced by immune mediators that can be processed by non-capsaicin fibers of the vagal pathway (Bret-Dibat et al., 1995, 1997; Berthoud et al., 1997; Zafra et al., 2004) or by splanchnic nerves and the humoral pathway (Cervero, 1994; Dantzer et al., 2000; Goehler et al., 2000; Langhans, 2000; Schwartz, 2002; Zafra et al., 2004). In other words, the absence of afferent signals required for satiation processes (eliminated by capsaicin) may increase food consumption, while the nociceptive information derived from the surgery would counteract this nutritional effect (Zafra et al., 2004). This could inhibit food intake during the initial periods (60 or 90 min) but would permit a higher intake over time (24 h) (see Fig. 4). In fact, the food intake differences observed in the controls for both experiments (capsaicin control, 84.33% *vs.* LPbNe control, 47.01%) may be attributable to the differential effects induced by the two surgical procedures (peripheral *vs.* central). Thus, body weight was only decreased in the animals that underwent peripheral surgery (capsaicin-treated group and its control group, see Fig. 3).

The importance of the vagal-LPbNe axis has been demonstrated in relation to other nutritional processes. Some authors reported that the LPbNe, among other nuclei, is involved in the nutritional effects generated by the peripheral action of cholecystokinin, which would act *via* the vagal pathway (Li and Rowland, 1995; Elmquist et al., 1997; 1998; Trifunovic and Reilly, 2001). Moreover, LPbNe lesions were found in *c-fos* studies to significantly reduce the activation of prosencephalic structures that process the action of fenfluramine, such as the central nucleus of the amygdala, and to reduce the anorexic effect induced by this drug (Li et al., 1994).

In the same line, the activation of LPbNe, among other nuclei, has also been reported after the peripheral administration of drugs that generate glucose deprivation (e.g. 2,5-anhydro-D-mannitol) and after the intraduodenal administration of glucose (Ritter et al., 1994; Wang et al., 1999), while vagotomy interrupted these effects (Ritter and Dinh 1992; Calingasan and Ritter, 1993; Ritter et al., 1994).

In addition, some peptides that regulate macronutrient consumption, such as NP-Y (carbohydrates), galanin (fats), and growth hormone releasing factor (proteins), are processed by the LPbNe, among other nuclei (Petrov et al., 1992; Krukoff et al., 1993; Veening et al., 1998; Koegler et al., 1999; Bray, 2000).

Finally, the vagal-LPbNe pathway also appears to be essential in certain regulatory behavioral manifestations that require rapid processing of information from the digestive system. Thus, in agreement with the present findings, vagotomies (Arnedo et al., 1990; Zafra et al., 2006, 2007, 2017a), large lesions of the entire LPbN area (Reilly and Trifunovic, 2000a; b; 2001), and specific lesions of the LPbNe subnucleus (Mediavilla

et al., 2000; Zafra et al., 2002) interrupt discriminative taste learning that requires the rapid processing of visceral information produced by the intragastric administration of rewarding (Zafra et al., 2002; 2007) or aversive (Arnedo et al., 1990; Mediavilla et al., 2000) substances. Moreover, the LPbN area is a brainstem region that participates in both rewarding (Zafra et al., 2002) and aversive (Agüero et al., 1993a, b; Carter et al., 2015; Mediavilla et al., 2000; Zafra et al., 2005) nutritional processes.

The vagus nerve is known to project to the nucleus of the solitary tract (NTS), which relays the information to the LPbNe (Herbert et al., 1990). According to a recent report by our group, the gelatinous subnucleus of the NTS appears to act as an intermediate relay between the vagus nerve and the LPbNe (Zafra et al., 2017b, c), and therefore may form part of this pathway.

CONCLUSION

The results of this study demonstrate that the short-term intake of solid food and sucrose solution is increased both by perivagal capsaicin-induced axotomy of afferent vagal pathways and by lesioning of the LPbNe subnucleus, one of the brain centers towards which the vagus nerve projects, presumably due to interruption of the rapid vagal visceral-sensory signals required for physiological satiation processes.

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