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# Disruption of re-intake after partial withdrawal of gastric food contents in rats lesioned in the gelatinous part of the nucleus of the solitary tract

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ABSTRACT

Sensory information from the upper gastrointestinal tract is critical in food intake regulation. Signals from different levels of the digestive system are processed to the brain, among other systems, *via* the vagus nerve, which mainly projects towards the nucleus of the solitary tract (NST). The objective of this study was to analyze the participation of the gelatinous part (SolG) of the NST in short-term food intake. One-third of the stomach food content was withdrawn at 5 min after the end of a meal, and food was then available *ad libitum* for different time periods. SolG-lesioned and control animals ingested a similar amount of the initial liquid meal, but the former consumed significantly smaller amounts and failed to compensate for the food deficit, whereas the controls re-ingested virtually the same amount as extracted. These data suggest that the SolG, as in the case of related anatomical structures such as the vagus nerve or external lateral parabrachial subnucleus, may be relevant in particular circumstances that require the rapid processing of vagal-related food intake adjustment associated to the upper gastrointestinal tract.
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## 1. Introduction

Study of the mechanisms involved in the control of food intake (meal size) has been of special interest over the years due to their potential importance in the treatment of obesity, a major public health problem in developed countries (Page, Symonds, Peiris, Blackshaw, & Young, 2012; Folgueira, Seoane, & Casanueva, 2014; D'Agostino et al., 2016).

Information generated by food in the upper gastrointestinal tract appears to be crucial in meal size control (Powley & Phillips, 2004; Roman, Derkach, & Palmiter, 2016; Schwartz, 2006). These mechanical (volumetric) and chemical data are largely transmitted to the brain *via* the vagus nerve (Phillips & Powley, 1998; Zafra, Molina, & Puerto, 2003), whose afferents project almost exclusively, and with a certain viscerotopic organization, to the nucleus of the solitary tract (NST) (Altschuler, Bao, Bieger, Hopkins, & Miselis, 1989; Barraco, el-Ridi, Ergene, Parizon, & Bradley, 1992; Gieroba & Blessing, 1994), a gateway of visceral signals to the brain (D'Agostino et al., 2016; Roman et al., 2016). It has been demonstrated that the densest concentration of gastric vagal afferents is in the lateral portion of the dorsomedial part of the intermediate-caudal region of the NST, known as the gelatinous subnucleus (SolG) (Altschuler et al., 1989; Barraco et al., 1992; Herbert, Moga, & Saper, 1990; Rinaman, Card, Schwaber, & Miselis, 1989; Shapiro & Miselis, 1985). However, in contrast to other parts of the dorsomedial subnucleus, the SolG receives few intestinal projections, which are preferentially distributed in more caudal regions (Barraco et al., 1992; Zhang et al., 1995, 1992; Zittel, De Giorgio, Sternini, & Raybould, 1994).

*C*-fos activity has been observed in specific subnuclei of the intermediate-caudal region of the NST (NSTic) after the normal intake of a meal (Emond, Schwartz, & Moran, 2001; Fraser & Davison, 1993; Gaykema et al., 2009; Olson et al., 1993; Rinaman, Baker, Hoffman, Stricker, & Verbalis, 1998), after the direct administration of nutrients in different digestive segments (Emond et al., 2001; Phifer & Berthoud, 1998; Wang, Cardin, Martínez, Taché, & Lloyd, 1999; Yamamoto & Sawa, 2000a,b; Zittel et al., 1994; Mönnikes et al., 1997) and in response to certain intake-related stimuli, including gastric distension (Gonzalez, Sharp, & Deutsch, 1986; Olson et al., 1993; Fraser et al., 1995; Zhang et al.,

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1995; Willing & Berthoud, 1997; Emond et al., 2001; Mazda, Yamamoto, Fujimura, & Fujimiya, 2004; van de Wall, Duffy, & Ritter, 2005), intestinal distension (Zhang, Fogel, & Renehan, 1992, 1995, 1998), and peripheral peptide secretion/administration (Fraser & Davison, 1992; Olson et al., 1993; Yang et al., 2004; Li & Rowland, 1995; van de Wall et al., 2005). This effect has even been recorded in response to aversive visceral stimuli (Mediavilla, Bernal, & Puerto, 2007, Yamamoto & Sawa, 2000 a,b). In many of these cases, NST activation can be abolished by chemical or surgical lesions of vagal afferents (Fraser & Davison, 1992; Li & Rowland, 1995; Mönnikes et al., 1997; Yamamoto & Sawa, 2000a; Mazda et al., 2004; Yang et al., 2004; van de Wall et al., 2005).

Hence, the NST is known to be involved in processes related to nutrient intake, and lesions of certain NST subnuclei have been found to trigger the overconsumption of preferred foods (South & Ritter, 1983), to reduce nutrient intake (Menani, Colombari, Talman, & Johnson, 1996), to block the effects on intake of some food-related drugs (Treece, Ritter, & Burns, 2000), and to interrupt taste aversion learning (Mediavilla, Bernal, Mahía, & Puerto, 2011).

With this background, the objective of the present study was to examine the relevance in short-term food intake regulation of the SolG, one of the subnuclei of the intermediate-caudal region of the NST, as noted above, by investigating re-intake behavior after the removal of part of the gastric content immediately after ending a test meal (satiation). In these conditions, neurologically intact animals habitually consume food until they recover approximately the same amount as extracted (Snowdon, 1970; Davis & Campbell, 1973; Deutsch, Young, & Kalogeris, 1978; Zafra, Molina, & Puerto, 2016a,b). Our hypothesis was that animals with SolG lesions would consume a significantly lower amount in comparison to non-lesioned controls and, as shown in related studies (Zafra et al., 2016a,b), would be unable to compensate for the deficit created, because they lack the vagal information required for the correct regulation of this behavior.

## 2. Materials and methods

### 2.1. Subjects

Twenty-four adult male Wistar rats (286–334 g at time of surgery), randomly assigned to two groups (SolG-lesioned group: N = 12; control sham-lesioned group: N = 12), were used in this experiment. The animals were individually housed in 30 × 15 × 30 cm methacrylate cages with free access to water and pellet stock diet (Panlab, S.L. Barcelona). The laboratory was maintained under a 12/12 h light-dark cycle (lights on 08:00 h) and at a temperature of 22 ± 1 °C. All experimental procedures took place during light periods and were conducted in accordance with the Animal Care and Use Guidelines established by European Community Council Directive (86/609/CEE) and Spanish legislation (Royal Law 1201/2005). All efforts were made to minimize animal suffering and the number of animals used.

### 2.2. Surgical procedure

#### 2.2.1. SolG lesions

Surgery was carried out under general anesthesia with sodium pentothal (50 mg/kg, ip; B Braun Medical S.A. Barcelona, Spain) with the animals placed in a stereotaxic unit (Stoelting Co. Stereotaxic 51.600). An incision approximately 1.5 cm in length was made in the upper area of the cranium, connective tissue adhered to the cranium was removed, and two small trephine holes were drilled at the anteroposterior and lateral coordinates corresponding to the SolG. The *dura mater* was then sectioned, and a 00 monopolar stainless steel electrode (approximately 200 μm in diameter and insulated throughout its length except at the tip) was introduced until it reached the dorsoventral coordinate. The electric circuit was then completed by a mass electrode placed in the periphery of the animal, and a cathodic electric current (0.3 mA) was bilaterally applied for 10 s with a DCML-5 lesion-maker (Grass Instruments Corp., Quincy, Mass, USA). The anatomical coordinates (interaural references) for the SolG, obtained from the Paxinos and Watson stereotaxic atlas (1996), were: anterior/posterior (AP) = −4.3 mm; lateral (L) = ± 0.9 mm; and dorsoventral (V) = +2.3 mm.

All of the above steps were followed for the sham lesion control group except that a dorsoventral coordinate of +2.9 mm was used and no current was applied.

#### 2.2.2. Intragastric catheter

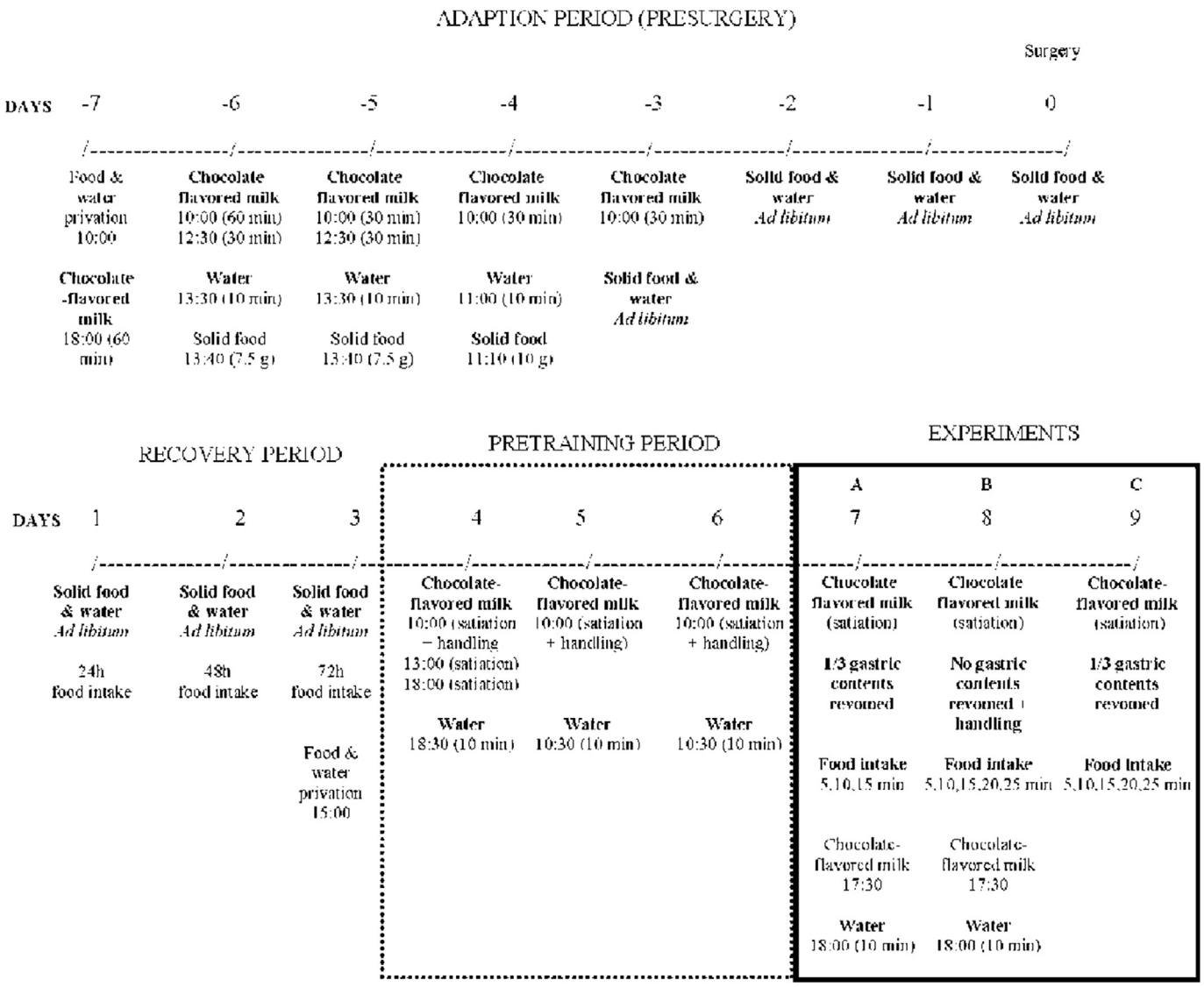
After the brain surgery, an intragastric catheter was implanted following a procedure reported elsewhere (Deutsch & Koopmans, 1973; Zafra et al., 2016a,b). A laparatomy of approximately 3 cm was performed, and the stomach was carefully pulled out from the abdominal cavity. An incision of approximately 2 cm was made in the cardia region at the greater curvature, through which a silastic tube was inserted (ID = 1.0 mm; OD = 2.0 mm). Around the end of the silastic tube, a small silicone protuberance was performed to prevent outward displacement of the catheter once the incision was closed around it. Closure was accomplished with a suture around the stomach tissue surrounding the catheter at its insertion site. In addition, the catheter was anchored to the stomach by making a suture point on the surface of the gastric tissue with the remaining suture thread. The exteriorized organs were kept continuously irrigated with isotonic physiological serum (Apiroserum. Lab. YBIS, Madrid, Spain) throughout this procedure. Next, the stomach was returned to the gastric cavity in its original position, and the catheter was routed through the abdominal muscle wall and tunneled subcutaneously to the dorsal surface behind the neck. Stitching was performed as needed to close the wounds, the catheter was capped to avoid gastric content leaking, and silicone was applied around the tip of the catheter to prevent its displacement within the subcutaneous tunnel. As prophylactic measures against infection, povidone iodine (Betadine, Asta Médica, Madrid, Spain) was topically applied to the wounds, and 0.1 cc penicillin (10,000 U; Penilevel Retard. Lab., Level, S.A. Barcelona) was intramuscularly injected.

### 2.3. Behavioral procedure

The behavioral procedure began seven days before the surgery (see Table 1). During the first five days of this period (Table 1: days −7 to −3), rats were adapted to consume a liquid diet (chocolate-flavored milk, Puleva Food, S.L., Granada; 100 ml contains 12.2 g carbohydrates, 2.2 g fat, and 3 g protein; total energy = 81 Kcal). On the morning of the first day of this 5-day period (10:00), animals were deprived of food and water and then, at the end of the afternoon (18:00), were presented with the liquid diet for the first time (during 1 h). On days −6 and −5, the diet was offered at 10:00 and 12:30 for 30 min (except for the first session on day −6, when it was offered for 60 min). On days −4 and −3, this diet was offered for only 30 min (at 10:00). On days −6 to −4, water was offered for 10 min at around 30 min after finishing the food ingestion session, followed by a pellet stock diet (7.5 g on days −6 and −5; 10 g on day −4) (Table 1). On days −3 to −1, solid food (pellet stock diet) and water were available *ad libitum* (on day −3 after consumption of the liquid diet).

On day 0, the rats underwent surgery (SolG-lesion/Sham-lesion and intragastric catheter). During this day and the next three days (recovery period: days 1–3 in Table 1), a diet of solid food (pellet stock diet) and tap water was available *ad libitum*, and the amount of

**Table 1**
Time course of experiment.




food consumed during 24, 48, and 72 h post-surgery was quantified.

Next, animals were again deprived of access to food and water (at 15:00 h on day 3) and underwent a 3-day pre-training period (days 4–6 in Table 1) for re-adaptation to the liquid diet. On the first day, they were offered chocolate-flavored milk twice in the morning (10:00 and 13:00) and once in the afternoon (18:00) until satiation (5 min without consuming food); on the following two days, milk was presented only once in the morning (10:00) until satiation. After the first ingestion session (morning session), animals were removed from their cage and subjected to a handling session that simulated the experimental procedure. Animals were offered water for 10 min at the end of each pre-training session (after the afternoon session on day 4).

### 2.4. Experiment

This stage followed the 3-day pre-training period, and three experiments (A, B, and C) were performed on consecutive days (days 7, 8, and 9 in Table 1).

#### 2.4.1. Experiment A

Experiment A began by offering a burette with chocolate-flavored milk that the animals consumed until satiation (defined by 5 min without intake), recording the intake duration. After 5 min without consuming, animals were removed from their cages, and one-third of the amount of food ingested was withdrawn from the stomach (this procedure usually took 0.5–1 min). They were subsequently returned to their cages, where the liquid diet remained available *ad libitum*. Re-intake was quantified every 5 min during a 15-min period.

In order to ensure adequate nourishment of the animals, the chocolate-flavored milk was presented again in the afternoon for 30 min, followed, as in previous studies (Zafra et al., 2016a,b), by the availability of water for 10 min; hence, there was a 16-h period of food and water deprivation before the next morning session.

#### 2.4.2. Experiment B

On the next day, experiment B was conducted with the same animals. This experiment was identical to experiment A except

that: (1) no gastric food contents were removed (after the 5-min food deprivation period, animals were subjected to a brief handling period and returned to their cages); (2) re-intake was quantified every 5 min but now during a 25-min period (at 5, 10, 15, 20, and 25 min).

The chocolate-flavored milk was again presented in the afternoon for 30 min (to ensure adequate nourishment) and water for 10 min.

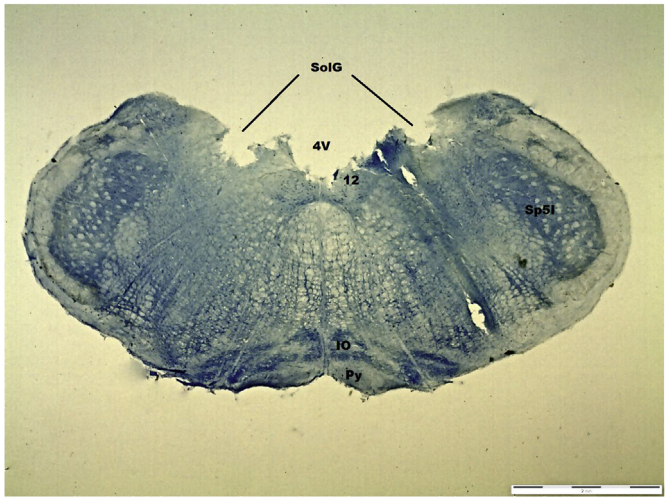



**Fig. 1.** Histological preparation stained with Cresyl Violet, showing the localization of the SolG lesion in a representative animal of this experiment (12: hypoglossal nucleus; 4V: 4th ventricle; IO: inferior olive; SolG: nucleus of solitary tract, gelatinous; Sp5I: spinal 5 nucleus, interpolar part; Py: pyramidal tract).

### 2.4.3. Experiment C

Experiment C was identical to experiment A (with the same animals) except that the re-intake was quantified every 5 min during a 25-min period.

## 2.5. Histology

At the end of the experiment C, SolG-lesioned animals were deeply anesthetized with an overdose of sodium pentothal and intracardially perfused with isotonic saline and 10% formaldehyde. Brains were removed and stored in formaldehyde (10%) for at least one week before being cut into 70-μ sections with a cryostat (Microm HM 550, Microm International GmbH, Walldorf). Sections were mounted, stained with Cresyl Violet, examined under a light microscope (stereoscopic microscope UMZ-4F; Olympus, Tokyo, Japan), and microphotographed (VMZ-4F stereoscopic magnifying glass and PM-6 camera, Olympus, Tokyo, Japan). Fig. 1 depicts an example of the histological study.

## 2.6. Statistical analyses

Statistica 5.1 program (Statsoft, Tulsa, USA) was used for statistical analyses. Results were analyzed by one- two- and three-way repeated-measures ANOVA. Significant effects were analyzed by means of a post-hoc Tukey test. Food re-intake analysis (experiments A, B and C) considered accumulated direct data at the different time points (see Table 2). The cumulative re-intake at the final time points (15 min in experiment A, 25 min in experiments B

**Table 2**
Experimental data (cumulative re-intake).

| PRETRAINING PERIOD | | |
|---|---|---|
| | SolG | Sham |
| Day 4: Chocolate flavored milk intake (ml), 10:00 session | 6.00 | 7.00 |
| Day 4: Chocolate flavored milk intake (ml), 13:00 session | 4.53 | 5.27 |
| Day 4: Chocolate flavored milk intake (ml), 18:00 session | 6.96 | 7.77 |
| Day 5: Chocolate flavored milk intake (ml), 10:00 session | 9.10 | 9.90 |
| Day 6: Chocolate flavored milk intake (ml), 10:00 session | 9.33 | 10.77 |
| **EXPERIMENT A** | | |
| | SolG | Sham |
| Day 7: Chocolate flavored milk intake (ml), satiation | 10.75 | 11.70 |
| Day 7: Extracted chocolate flavored milk (ml), 1/3 | 3.55 | 3.88 |
| Day 7: Chocolate flavored milk re-intake (ml), 5 min | 0.54 | 0.52 |
| Day 7: Chocolate flavored milk re-intake (ml), 10 min | 0.76 | 1.18 |
| Day 7: Chocolate flavored milk re-intake (ml), 15 min | 1.11 | 1.87 |
| Day 7: Chocolate flavored milk intake (ml), afternoon maintenance session | 11.80 | 12.79 |
| **EXPERIMENT B** | | |
| | SolG | Sham |
| Day 8: Chocolate flavored milk intake (ml), satiation | 12.75 | 13.07 |
| Day 8: Chocolate flavored milk re-intake (ml), 5 min | 0.00 | 0.08 |
| Day 8: Chocolate flavored milk re-intake (ml), 10 min | 0.00 | 0.08 |
| Day 8: Chocolate flavored milk re-intake (ml), 15 min | 0.00 | 0.30 |
| Day 8: Chocolate flavored milk re-intake (ml), 20 min | 0.00 | 0.51 |
| Day 8: Chocolate flavored milk re-intake (ml), 25 min | 0.00 | 0.69 |
| Day 8: Chocolate flavored milk intake (ml), afternoon maintenance session | 12.75 | 14.31 |
| **EXPERIMENT C** | | |
| | SolG | Sham |
| Day 7: Chocolate flavored milk intake (ml), satiation | 13.04 | 14.53 |
| Day 7: Extracted chocolate flavored milk (ml), 1/3 | 4.33 | 4.81 |
| Day 7: Chocolate flavored milk re-intake (ml), 5 min | 0.21 | 0.96 |
| Day 7: Chocolate flavored milk re-intake (ml), 10 min | 0.29 | 1.62 |
| Day 7: Chocolate flavored milk re-intake (ml), 15 min | 0.55 | 2.19 |
| Day 7: Chocolate flavored milk re-intake (ml), 20 min | 1.11 | 3.17 |
| Day 7: Chocolate flavored milk re-intake (ml), 25 min | 1.89 | 4.70 |

and C) was also analyzed as percentage of meal size. All data were expressed as means ± standard error of the mean (SEM), and $p < 0.05$ was considered significant.

## 3. Results

One animal from the control group was excluded for catheter detachment (statistical analyses only included the data from 12 SolG-lesioned animals and 11 controls).

### 3.1. Food intake in the adaptation period (pre-surgery)

Two-way ANOVA [group (SolG-lesioned vs Sham-lesioned) x intake sessions] results showed no significant difference between the groups in the intake of chocolate-flavored milk [$F(1,21) = 0.25$, $p < 0.61$] or solid food [$F(1,21) = 0.99$, $p < 0.32$] before the surgery.

### 3.2. Food intake during the recovery period

Two-way ANOVA (group x days) results showed no significant differences between groups in solid food intake during the three days post-surgery [$F(1,21) = 0.64$, $p < 0.43$].

### 3.3. Food intake during the pretraining period

There were no between-group differences in chocolate-flavored milk intake during pretraining sessions [$F(1,21) = 2.13$; $p < 0.15$; two-way ANOVA: group x intake sessions].

### 3.4. Food intake in experiments A, B, and C

#### 3.4.1. Chocolate-flavored milk intake pre-extraction

Two-way ANOVA (group x meal size) results showed no between-group differences in the amount of food ingested before the extraction on any experimental day (A, B, C) [$F(1,21) = 0.61$, $p < 0.44$] and no differences in the time invested in intake [$F(1,21) = 0.59$, $p < 0.449$].

#### 3.4.2. Experiment A

Two-way ANOVA [group (SolG-lesioned vs. Sham-lesioned) x interval (extraction, re-intake at 5, 10, and 15 min)] showed no significant differences between groups [$F(1,21) = 1.33$, $p < 0.26$; see Fig. 2].

However, intragroup analysis showed significant differences between the extracted amount and the cumulative intake at each of the three time points in both the SolG-lesioned group [5 min: $p < 0.00016$; 10 min: $p < 0.00016$; 15 min: $p < 0.00016$; see Fig. 2] and the sham-lesioned control group [5 min: $p < 0.00016$; 10 min: $p < 0.00016$; 15 min: $p < 0.00016$; see Fig. 2].

#### 3.4.3. Experiment B

The total re-intakes of the SolG-lesioned and sham-lesioned groups were 0.0 ml and 0.69 ml, respectively (Table 2). Two-way ANOVA [group (SolG-lesioned vs. Sham-lesioned) x interval (extraction, cumulative re-intake at 5, 10, 15, 20, and 25 min)] showed that the effects of group [$F(1,21) = 6.92$, $p < 0.015$], interval [$F(5,105) = 4.33$, $p < 0.0012$], and group × interval interaction [$F(5,105) = 4.33$, $p < 0.012$] were all significant; however, it was not possible to analyze the interaction with the post-hoc Tukey test because the matrix was near-singular.

#### 3.4.4. Experiment C

Two-way ANOVA [group (SolG-lesioned vs Sham-lesioned) x interval (extraction, re-intake at 5, 10, 15, 20 and 25 min)] showed that the effects of group [$F(1,21) = 10.51$, $p < 0.0039$], interval [$F(5,105) = 51.45$, $p < 0.00001$], and group × interval interaction [$F(5,105) = 4.06$, $p < 0.002$] were all significant.

Post-hoc analysis found no significant between-group differences in the amounts extracted ($p < 0.99$), and the cumulative re-intake measured at 5 min ($p < 0.83$) or 10 min ($p < 0.087$), but significant between-group differences were found at 15 min ($p < 0.01$), 20 min ($p < 0.0003$), and 25 min ($p < 0.0001$).

In the SolG-lesioned group, a significant difference was found between the amounts extracted and the cumulative re-intake measured at all time points [5 min: $p < 0.00011$; 10 min: $p < 0.00011$; 15 min: $p < 0.00011$; 20 min: $p < 0.00011$; 25 min: $p < 0.00012$; see Fig. 3]. In the sham-lesioned control group, a significant difference was found at all time points except for the last one at 25 min [5 min: $p < 0.00011$; 10 min: $p < 0.00011$; 15 min: $p < 0.00011$; 20 min: $p < 0.013$; 25 min: $p < 1.0$; see Fig. 3].

#### 3.4.5. Experiment A vs. B

Three-way ANOVA [group (SolG-lesioned vs. sham-lesioned) x test days (A, B) x interval (extraction, cumulative re-intake at 5, 10, and 15 min)] showed no significant between-group differences [$F(1,21) = 2.08$, $p < 0.16$], whereas significance was found for the effects of days [$F(1,21) = 105.66$, $p < 0.00001$] and interval [$F(3,63) = 65.87$, $p < 0.00001$].

*3.4.5.1. Chocolate-flavored milk re-intake at 15 min post-extraction as percentage of meal size: experiments A and B.* Three-way ANOVA [group (SolG-lesioned vs. sham-lesioned) x days (A, B) x interval (extraction vs. re-intake at 15 min)] showed no significant between-group differences [$F(1,21) = 1.6$, $p < 0.21$]; however, a significant effect was found for days [$F(1,21) = 249.9$, $p < 0.000001$]

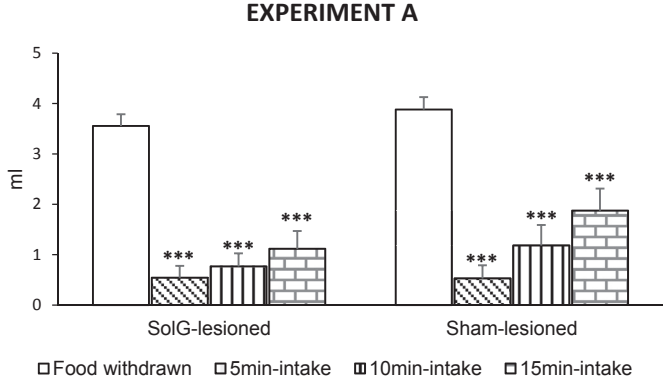



**Fig. 2.** Mean intake (cumulative data) of liquid diet (chocolate-flavored milk) by animals in experiment A (SolG-lesioned and sham-lesioned) at 5, 10, and 15 min (***: 0.001).

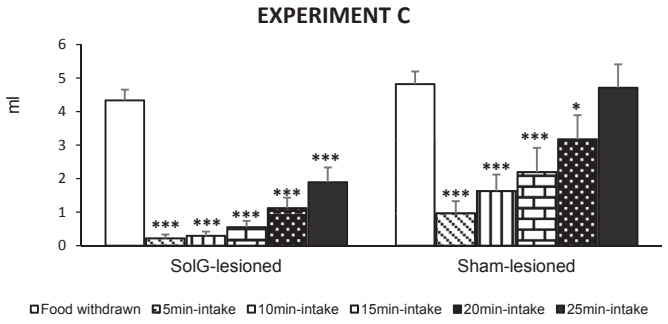



**Fig. 3.** Mean intake (cumulative data) of liquid diet (chocolate-flavored milk) by animals in experiment C (SolG-lesioned and sham-lesioned) in the re-intake test at 5, 10, 15, 20, and 25 min (*: 0.05; ***: 0.001).

and interval [$F(1,21) = 40.57$, $p < 0.000003$]. In experiment A, SolG-lesioned animals had reingested 35.1% of the extracted food at 15 min, by which time the sham-lesioned animals had reingested 47.6%.

*3.4.6. Experiment B vs. experiment C*

Three-way ANOVA [group (SolG-lesioned vs sham-lesioned) x test days (B, C) x interval (extraction, cumulative re-intake at 5, 10, 15, 20, and 25 min)] revealed that the effects of group [$F(1,21) = 15.59$, $p < 0.0007$], days [$F(1,21) = 64.47$, $p < 0.00001$], interval [$F(5,105) = 43.68$, $p < 0.00001$], interaction group x days [$F(1,21) = 6{,}02$, $p < 0.02$], and interaction group x interval [$F(5,105) = 5.75$, $p < 0.00009$] were all significant, whereas significance was not reached for group x days x interval [$F(5,105) = 2.01$, $p < 0.08$].

*3.4.6.1. Cumulative re-intake of chocolate-flavored milk at 25 min post-extraction as percentage of meal size: experiments B and C.* In a three-way ANOVA [group (SolG-lesioned vs. sham-lesioned) x days (B, C) x interval (extraction vs. re-intake at 25 min)], significant effects were found for group [$F(1,21) = 14.08$, $p < 0.0011$], days [$F(1,21) = 393.46$, $p < 0.000001$], and for group x days $\times$ interval interaction [$F(1,21) = 5.03$, $p < 0.035$].

Post-hoc analysis found no significant between-group differences in cumulative intake at 25 min ($p < 0.414$) in experiment B, but significant differences were observed at this time in experiment C ($p < 0.00018$). In experiment C, no difference was found between the amounts extracted and re-ingested at 25 min in the sham-lesioned group ($p < 0.999$) but a significant difference was observed in the SolG-lesioned group ($p < 0.00016$). Thus, these animals had reingested only 44.02% of the extracted food volume at 25 min, by which time the sham-lesioned animals had re-ingested 99.4%.

*3.5. Food intake afternoon maintenance sessions*

The groups did not significantly differ in the intake of chocolate-flavored milk during the maintenance session at 17:30 in either experiment A [$F(1,21) = 0.75$ $p < 0.39$; one-way ANOVA; 11.8 vs. 12.79] or experiment B [$F(1,21) = 2.19$, $p < 0.15$; one-way ANOVA; 12.75 vs. 14.31].

## 4. Discussion

Results obtained in experiment C of this study demonstrate that lesions in the SolG of the NST interfere with the usual re-intake behavior observed in neurologically intact animals following extraction of one third of gastric contents immediately after a meal. Thus, the short-term post-extraction consumption was significantly lower in the lesioned animals than in the controls, and the former had not compensated for the deficit at 25 min, whereas the control animals had consumed around the same amount as extracted (Fig. 3).

No significant between-group differences were observed in experiment A (Fig. 2), when animals only had 15 min for post-extraction re-intake, which the results in controls suggest is not long enough to fully compensate for the extracted food. Hence, the physiological changes induced by extraction of the nutrient appear to have become relevant in the subsequent 10 min. Thus, the neurologically intact animals appeared to process the deficit created and compensate for the lost nutrients in the interval between 15 and 25 min post-extraction [In fact, they had recovered around 47% of the extracted food volume after 15 min (experiment A) and almost 100% after 25 min (experiment C)]. In contrast, this deficit does not appear to be processed by the SolG-lesioned animals, which did not compensate during this 25-min period for the food extracted, re-ingesting only 44% of its volume.

The results of experiment B demonstrate the effect of the lesion alone on the experimental animals. Thus, whereas the sham-lesioned group started to consume small amounts of food during the final re-intake intervals, there was absolutely no intake by the lesioned animals at this time. These between-group differences were much more marked in experiment C, in which the animals underwent both the lesion and the removal of one-third of their gastric content, which these animals must detect but for which they cannot compensate.

As in the two previous studies in this experimental series (Zafra et al., 2016a,b), the animals had access to water for 10 min during the pretraining period and in Experiments A-C. This intake was offered after the afternoon "hydration" session at 18:00, during which the rats consumed chocolate-flavored milk but little or no water.

The fact that the experimental animals were hungry and thirsty may suggest that the deficit induced by SolG lesion cannot be conclusively attributed to a deficit in nutrient regulation. However, although researchers such as Snowdon (1970) and Davis and Campbell (1973) offered the animals water *ad libitum*, their food intakes were highly similar to the intake of flavored milk by our water-restricted animals. This is despite the longer re-intake time allowed in their studies (30–45 min), potentially increasing gastric emptying and favoring higher intake, which did not prove to be the case.

In previous studies using highly palatable nutrients, such as chocolate-flavored milk, damage in NST regions adjacent to the *area postrema* was found to produce overconsumption of this type of food (South & Ritter, 1983). However, the SolG lesion does not seem to have affected the consumption of this food either positively or negatively in our experiment, with no between-group differences in its consumption at any time point (before or after surgery). SolG lesions also appear to exert no effect on solid food intake, which was also reported to be unaffected by in more caudal subnuclei of the NST (Menani et al., 1996).

Likewise, the present results suggest that SolG-lesioned animals regulate the initial meal size in a normal manner, given that no between-group differences were found in the amount or duration of pre-extraction food intake in any experiment. Thus, regulation disturbances only arise when there is a need for rapid detection of the signals generated by partial food extraction (e.g., reduced gastric volume/content, modification of duodenal emptying, hormone release). These signals were adequately processed by the control animals, which adapted their behavior to compensate for the deficit created by food removal.

Given that partial food extraction can modify gastric emptying into the small intestine (Kaplan, Siemers, & Grill, 1994), the origin of the volumetric and/or chemical signals interrupted by SolG lesions can be either gastric or post-gastric (Powley & Phillips, 2004; Sengupta & Gebhart, 1994). However, it has been demonstrated that the SolG is the NST subnucleus with the highest concentration of gastric afferents (Barraco et al., 1992; Zhang et al., 1995; Rinaman et al., 1989; Shapiro & Miselis, 1985; Willing & Berthoud, 1997) and has a significantly lower fiber supply from lower levels of the digestive system (Altschuler et al., 1989; Zhang et al., 1995). It therefore appears likely that the information affected by the SolG lesion and involved in re-intake behavior is of gastric origin.

Several researchers have reported that gastric information related to short-term nutrition regulation is mainly volumetric (Mathis, Moran, & Schwartz, 1998; Phillips & Powley, 1996; Powley & Phillips, 2004). Thus, in a study using single unit recordings, Zhang et al. (1995) reported that the SolG processes mechanical information and that most of its neurons are only sensitive to stomach distension, with only a few neurons that respond to both

gastric and duodenal distension; however, these data were not replicated in a *c*-fos study (Fraser et al., 1995).

Our finding that neurologically intact animals re-ingest around the same amount as removed when again allowed access to the test food is in agreement with various studies (Snowdon, 1970; Davis & Campbell, 1973; Deutsch et al., 1978; Zafra et al., 2016a,b). Our results in SolG-lesioned animals are also consistent with recent findings. Thus, animals vagally deafferented using perivagal capsaicin (Zafra et al., 2016a) and those with lesions of the pontine external lateral parabrachial subnucleus (PBLe) (Zafra, Agüera, Simón, Molina, & Puerto, 2016b) also show impaired regulation of re-intake behavior, although the temporal patterns slightly varied, likely due to differences in the degree of interruption (vagus nerve [major] *versus* SolG [minor]) of the flow of information from the gastrointestinal system.

Hence, the SolG, alongside vagal afferents (capsaicin-sensitive) and the PBLe, may be part of a neural pathway that processes information from the upper gastrointestinal tract and is necessary for the correct expression of re-intake behavior. Accordingly, as noted above, it has been demonstrated that the SolG is one of the main objectives of gastric vagal afferents (Altschuler et al., 1989; Barraco et al., 1992; Shapiro & Miselis, 1985) and, along with the rest of the dorsomedial NST of which it is part, it is a relay of fine vagal afferents (Torrealba & Calderón, 1990). These fibers are of the type that is lesioned by capsaicin (Holzer, 1991; Jancsó, 1978), and damage of small ganglion cells by capsaicin has been found to produce axonal degeneration in the SolG, among other regions (Jancsó & Király, 1980).

In this line, Herbert et al. (1990) demonstrated that the projections of the SolG do not differ from those of the rest of the dorsomedial region of the NST. They mainly project towards the outer zone of the external lateral parabrachial nucleus (Acuña-Goycolea, Fuentealba, & Torrealba, 2000; Herbert et al., 1990), which is connected to structures known to be involved in food intake, such as the paraventricular and lateral hypothalamic nuclei or the central nucleus of the amygdala (Bernard, Alden, & Besson, 1993; Bester, Besson, & Bernard, 1997; Fulwiler & Saper, 1984).

There have been reports on the involvement of this potential vagal-NSTic-PBLe processing pathway in other nutrition-related processes. Thus, the presence of different nutrients in the gastric cavity was found to induce *c*-fos expression in both intermediate-caudal NST subnuclei (e.g., dorsomedial) and the PBLe, among other regions (Emond et al., 2001; Yamamoto & Sawa, 2000a,b). This dual activation has also been observed after administration of pharmacological (methyl palmoxyrate, 2,5-Anhydro-D-mannitol, dexfenfluramine) or endocrine (cholecystokinin, bombesin, secretin) agents, which positively or negatively affect food intake [Li & Rowland, 1995, 1996; Horn & Friedman, 1998a,b; Horn, Tordoff, & Friedman, 2001; Yang et al., 2004]. Truncal vagotomy or perivagal capsaicin treatment has been found to abolish or attenuate these neuronal activations and/or intake effects [Smith, Jerome, Cushin, Eterno, & Simansky, 1981; Ladenheim and Ritter, 1991; Ritter, Dinh, & Friedman, 1994; Li & Rowland, 1995; Yamamoto & Sawa, 2000a; Horn et al., 2001; Yang et al., 2004].

The relevance of this rapid processing pathway has also been revealed in taste discrimination learning studies that require the immediate processing of either rewarding [Zafra, Simón, Molina, & Puerto, 2002, 2007] or aversive [Arnedo, Gallo, Agüero, & Puerto, 1991; Mediavilla, Molina, & Puerto, 2000; Zafra, Prados, Molina, & Puerto, 2006] visceral stimuli. Thus, it has been observed that this learning modality requires the integrity of the vagus nerve [Arnedo et al., 1991; Zafra et al., 2006, 2007], the NST [Mediavilla et al., 2011], and the LPBe [Mediavilla et al., 2000; Zafra et al., 2002].

In conclusion, the vagal-SolG-PBLe processing pathway may be especially important in circumstances that require the rapid detection and processing of visceral information. Neither the SolG nor the LPBe appears to be essential for satiation regulation under the conditions of our re-intake experiments, given that the volume of the initial pre-extraction intake did not differ from that of controls. However, both subnuclei are necessary when the rapid detection of gastrointestinal signals is required to compensate for a deficit (extraction of one-third of gastric contents at 5 min after satiation).

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