Appetite 118 (2017) 90–96

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Relevance of the nucleus of the solitary tract, gelatinous part, in learned preferences induced by intragastric nutrient administration

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ARTICLE INFO

Article history:
Received 14 May 2017
Received in revised form
2 August 2017
Accepted 3 August 2017
Available online 5 August 2017

Keywords:
Concurrent flavor learning
Predigested rewarding nutrients
Rapid visceral adjustments
NST dorsomedial region
Vagus-NST-LPBe axis

ABSTRACT

Food preferences have been investigated in Wistar rats utilizing a learned concurrent flavor preference behavioral procedure. Previous studies have demonstrated that the perivagal administration of neurotoxin capsaicin disrupts the learning of preferences induced by intragastric administration of rewarding nutrients (pre-digested milk). The vagus nerve projects almost exclusively towards the nucleus of the solitary tract (NST), a brain medullary gateway for visceral signals. The objective of this study was to investigate the participation of the lateral portion of the dorsomedial region, the gelatinous subnucleus (SolG), in the learning of a concurrent preference task. Results show that unlike neurologically intact animals, which learn this task correctly, animals lesioned in the gelatinous part of NST manifest a disruption of discrimination learning. Thus, intakes of the flavored stimulus paired with predigested liquid diet and of the flavored stimulus paired with physiological saline were virtually identical. However, SolG- and sham-lesioned groups consumed similar total amounts of both flavors. These findings suggest that SolG, as a relay of the vagus nerve, along with its anatomical projection, the external lateral parabrachial subnucleus (LPBe), may constitute an anatomical axis that is important in the induction of concurrent flavor/side preferences. It also appears to be relevant in other behavioral processes that require rapid processing of information from the upper gastrointestinal tract.

1. Introduction

The behavior of organisms in selecting food is largely directed by learning mechanisms. Thus, a nutrient initially preferred by a subject can be actively rejected if the consequences of its consumption prove negative (Garcia, Hankins, & Rusiniak, 1974), even when this preference is innate (Sclafani & Ackroff, 2012).

Various behavioral procedures have been utilized to investigate food preferences in the laboratory (Zafra, Molina, & Puerto, 2007; Zafra, Simón, Molina, & Puerto, 2007), including "concurrent preference". This involves the presentation of two non-nutritive flavor stimuli for a short time period, during which consumption of one of the stimuli is associated with the concurrent intragastric administration of a rewarding nutritive stimulus, while intake of the other stimulus is associated with the intragastric injection of a non-caloric and innocuous product, e.g., physiological saline (PS). Accomplishment of this behavioral task theoretically requires rapid detection and transmission to the brain of the visceral stimulus to enable a correct association of the visceral stimulus with the corresponding flavor (Mediavilla, Molina, & Puerto, 2005; Zafra, Prados, Molina, & Puerto, 2006).

The neurobiological components of the rapid information transmission pathway required have been studied. Thus, it has been reported that the vagus nerve, a neuroanatomical structure capable of the sensory detection of visceral stimuli (Blackshaw, Grundy, & Scratcherd, 1987; Mei, 1985; Melone, 1986; Sengupta & Gebhart, 1994), is essential for the induction of learned concurrent preferences. This learning was found to be blocked by the perivagal application of capsaicin (Zafra, Molina et al., 2007), a neurotoxin that mainly destroys afferent fibers (Holzer, 1991; Jancsó, Király, Such, Joó, & Nagy, 1987).

The vagus nerve almost exclusively projects towards the medullary nucleus of the solitary tract (NST) (Altschuler, Bao, Bieger, Hopkins, & Miselis, 1989; Barraco, el-Ridi, Ergene, Parizon, & Bradley, 1992), a brain gateway for visceral signal processing (D'Agostino et al., 2016; Roman, Derkach, & Palmiter, 2016).

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http://dx.doi.org/10.1016/j.appet.2017.08.004
0195-6663/

Sensory information from abdominal viscera is topographically organized with relative anatomical segregation (Altschuler et al., 1989; Barraco et al., 1992). Specifically, vagal afferents from the stomach are densely concentrated in the lateral portion of the dorsomedial NST, the gelatinous nucleus (SolG) (Altschuler et al., 1989; Barraco et al., 1992; Herbert, Moga, & Saper, 1990; Rinaman & Schwartz, 2004; Rinaman, Card, Schwaber, & Miselis, 1989; Shapiro & Miselis, 1985; Young, Cooper, & Blackshaw, 2008), while afferents from the duodenum and other segments of the small intestine are distributed elsewhere, mainly in more caudal and medial areas of the intermediate region (Altschuler et al., 1989; Barraco et al., 1992; Zhang, Fogel, & Renehan, 1992, Zhang, Fogel, & Renehan, 1995).

Given the need for rapid information processing in the concurrent procedure, we hypothesized that the digestive segments most likely to be involved in this learning procedure, i.e., those responsible for initial detection of the visceral stimulus, would be proximal segments, preferentially the stomach and duodenum. The SolG, unlike other dorsomedial regions or even other NST regions (Altschuler et al., 1989; Barraco et al., 1992; Shapiro & Miselis, 1985), almost exclusively receive gastric vagal afferents (Barraco et al., 1992; Zhang et al., 1995, 1992; Zittel, De Giorgio, Sternini, & Raybould, 1994).

With this background, the objective of the present study was to determine the participation of the SolG subnucleus of the NST in concurrent flavor learning, investigating whether it forms part of a pathway for the rapid transmission of rewarding nutritional information to the brain.

In flavor discrimination learning tasks, the intragastric administration of a wide variety of foods that are rewarding when orally consumed can frequently cause the rejection of associated flavors in subsequent presentations (Deutsch, Molina, & Puerto, 1976; Zafra, Simón et al., 2007). This has been attributed to the fact that the nutrients lack the physiological secretions that characterize the cephalic stage of digestion and are present during the oral consumption of these substances (Puerto, Deutsch, Molina, & Roll, 1976; Zafra, Molina, & Puerto, 2006; Zafra, Simón et al., 2007; Zafra et al., 2009). Accordingly, the present study used cephalic rewarding nutrients, obtained from donor animals after their oral consumption and likely to contain the corresponding cephalic secretions (Zafra, Simón et al., 2007).

2. Materials and methods

2.1. Subjects

Thirty-two adult male Wistar rats (weighing 286–335 g at time of surgery) were used in this experiment, randomly assigning 10 to a SolG-lesioned group, 10 to a control sham-lesioned group, and 12 to a donor group. All rats were individually housed in 30 × 15 × 30 cm methacrylate cages with *ad libitum* access to water and pelleted stock diet (Panlab, S.L. Barcelona). The laboratory was maintained under a 12/12 h light-dark cycle (lights on 08:00 h) at 22 ± 1 °C. All experiments were conducted during light periods 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). Every effort was made to minimize animal suffering and the number of animals used.

2.2. Surgical procedure

2.2.1. SolG lesions

Surgery was performed under general anesthesia with sodium pentothal (50 mg/kg, ip; B Braun Medical S.a. Barcelona, Spain). Once anesthetized, the animals were placed in a stereotaxic unit (Stoelting Co. Stereotaxic 51.600), and an incision of approximately 1.5 cm in length was made in the upper area of the cranium. Connective tissue was removed and two small trephine holes were drilled at the anteroposterior and lateral coordinates corresponding to the SolG. After sectioning the dura mater, a 00 monopolar stainless steel electrode with diameter of approximately 200 μm and insulated throughout its length (except at the tip) was introduced up to the dorsoventral coordinate. A cathodal electric current (0.3 mA) was bilaterally applied for 10 s using a DCML-5 lesion-maker (Grass Instruments Corp., Quincy, MA, USA). Anatomical coordinates for the SolG (interaural references), taken from the Paxinos and Watson (1996), were: anterior/posterior (AP) = −4.3 mm; lateral (L) = ±1.05 mm; and ventral (V) = +2.2 mm. All of the above steps were followed in the sham-lesion control group except that a vertical coordinate of +3.2 mm was used and no current was applied.

2.2.2. Intragastric catheters

After brain surgery (SolG-lesioned and sham-lesioned), two intragastric catheters were implanted following a previously reported procedure (Zafra, Molina, Puerto, 2016). After laparotomy of approximately 3 cm, 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, and a silastic tube (ID = 1.0 mm; OD = 2.0 mm) with small silicone protuberance around the end (to prevent outward catheter displacement) was inserted. The incision was closed with a suture around the stomach tissue surrounding the catheter at its insertion site. In addition, the catheter was anchored to the stomach using the remaining suture thread to make a suture point on the surface of the gastric tissue. This procedure was then repeated for implantation of the second catheter. The exteriorized organs were continuously irrigated with isotonic physiological saline (Apiroserum. Lab. YBIS, Madrid). The stomach was then returned to the gastric cavity in its original position, and the catheters were routed through the abdominal muscle wall and subcutaneously tunneled, one on each side of the animal, to the dorsal surface of the neck. Wounds were stitched, the catheters were capped to avoid gastric content leaking, and silicone was applied around the tip of the catheters to prevent its displacement within the subcutaneous tunnel. As prophylactic measures against infection, povidone iodine (Betadine, Asta Medica, 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. The same surgical procedure was performed to implant two intragastric catheters in the donor animals.

2.3. Behavioral procedure

2.3.1. Donor rats

Donor rats were placed in a different room and habituated to consume a liquid diet (Ideal Evaporated whole milk, 50% diluted. Nestlé, Barcelona, Spain) over four days following one day of food and water deprivation; 100 mL of this liquid diet contained 5.75 g carbohydrates, 3.93 g fat, and 3.93 g protein (total energy: 74.37 Kcal). During this habituation period, the liquid diet was offered for 3 h (from 10:00 to 13:00) followed by 10 min of access to water; in the first two days of this period, they received 7.5 g of solid food after removal of the water.

This four-day period was followed by a two-day period with solid food (pelleted stock diet) and tap water *ad libitum* before surgery (implantation of two intragastric catheters), maintaining this diet on the day of surgery and for the next six days. Animals were then offered a liquid diet (re-adaption period), following the same procedure as in the adaptation period (also offering 7.5 g of

solid food after removal of the water on the first day). This dietary regimen was maintained until the end of the study.

Gastric extractions were initiated three days before the experiment in order to habituate the animals to this practice (pretraining period; see Table 1).

2.3.2. SolG-lesioned and sham-lesioned rats

In the experimental animals (SolG-lesioned and sham-lesioned), the behavioral procedure began 9 days after the surgery. Subjects underwent a 3-day pretraining period (days 10–12) with water and food restrictions. During each pretraining day, animals were allowed to drink tap water from two 0.1-cc-graduated burettes simultaneously presented through the frontal orifices of the cages (to avoid place preferences). On the first two days, animals were offered water for 10 min on days 1 and 2 and for 7 min on day 3; 15 g of solid food was offered at 30 min after removal of the water (Table 1).

If the animal tended to consume water from only one of the burettes during the first two pretraining days, this burette was removed for the last 3 min of each session (to induce the animals to drink from the other burette). Consequently, virtually all animals had learned to alternate between the burettes on the third pretraining day.

The experiment began after the 3-day pretraining period (days 13–17). In each trial, which was limited to 7 min to minimize the involvement of post-absorptive factors, the rats were given a choice of two 0.1-cc-graduated burettes containing two flavored stimuli (0.5% strawberry [S] and 0.5% coconut [C] extract diluted in water, McCormick Co. Inc., San Francisco, CA, USA) that were simultaneously offered, maintaining the same positions of the burettes throughout the experiment (S on the left side of the animal and C on the right). Intake from one burette was paired with the intragastric injection of a partially digested (PD) liquid diet that had been pumped from the stomach of the donor rats (the food remained in the stomach of the donor rats for at least 30 min before its extraction), while intake from the other burette was paired with the intragastric injection of PS (0.9% NaCl, Apiroserum. Lab. YBIS, Madrid, Spain) administered *via* the second catheter (a graphic illustration of this procedure was previously published; Mediavilla et al., 2005). The PD liquid diet and PS were simultaneously injected each time the rats drank from the associated burette at a rate of 1 mL/1 mL ingested flavor stimulus.

Pairing of the PD liquid diet was counter-balanced in order to compensate for any natural flavor preferences of the subjects, so that half of the animals in each group received PD while drinking S and PS while drinking C, whereas the other half received PD while drinking C and PS while drinking S (diagram showing the balanced experimental conditions used in concurrent flavor preferences was published in Zafra, Simón, Molina, & Puerto, 2002). After 60 min, the SolG-lesioned and sham-lesioned control subjects were provided with 10 g of solid food; any food remaining at 19:00 h (15 h before the next session) was removed. This experimental procedure was repeated over the course of five trials (Table 1).

Table 1
Time course of experiment during the test phase.

Pretraining period (days 10–12)	Learned preferences (days 13–17)
Liquid diet	
10:00–13:00 h (gastric pumping-out)	10:00–13:00 h (gastric pumping-out)
Water	
13:30 (10 min)	13:30 (10 min)
Water (two burettes)	**Flavors (two burettes) + PD/PS (i.g.)**
10:00 h (10 min)	10:00 h (7 min)
Solid food	
10:30 (15 g)	11:00 (10 g)

Although there appears to be a predisposition in visceral/food-related learning to associate stimuli of visceral origin with gustatory-olfactory stimuli (Garcia et al., 1974; Mediavilla, Molina, & Puerto, 2001), concurrent learning was used in the present experiment, i.e., an implicit learning modality (Agüera, Bernal, & Puerto, 2016; Mediavilla et al., 2005), in which the flavor stimuli are always presented in the same location. It is therefore not possible to rule out an association with flavor or position clues in the learning acquisition (Mediavilla et al., 2001; Van Vort & Smith, 1983; de Araujo et al., 2008), and preferences are therefore referred to as flavor/side preferences in the present study.

2.4. Histology

At the end of the experiment, 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 their subsequent lamination (70 μ sections; 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, Tokio, Japan). Fig. 1 depicts the results of the histological study.

2.5. Statistical analyses

Statistica 5.1 program (Statsoft, Tulsa, OK, USA) was used for statistical analyses. Results [percentage of flavor intake after five trials/sessions of learning (day 5)] were analyzed using a two-way ANOVA. Mean total liquid intake (flavor paired with predigested liquid diet + flavor paired with physiological saline) was analyzed using direct data (two-way ANOVA).

3. Results

Four animals from the SolG-lesioned group and one from the control group were excluded due to catheter detachment; therefore, statistical analyses included data from 6 SolG-lesioned and 9 sham-lesioned animals.

3.1. Body weight

There were no significant differences between groups in body weight on the day of surgery [$F(1,13) = 1.0$, $p < 0.33$], during pretraining [$F(1,13) = 0.27$, $p < 0.6$], or during the experiment [$F(1,13) = 0.61$, $p < 0.446$].

3.2. Concurrent flavor/side preferences

Analysis of the concurrent preferences (two-way ANOVA % of total intake) shown by groups after five acquisition trials (on day 5) showed a significant (group x substance) interaction [$F(1,13) = 4,68$, $p < 0.049$].

The difference in flavor intake was not significant on any day (all p values > 0.05; Fig. 2) in the SolG-lesioned group but was significant on days 4 [$F(1,8) = 20.89$, $p < 0.0018$] and 5 [$F(1,8) = 15.92$, $p < 0.004$] in the sham-lesioned group, as depicted in Fig. 3.

Comparison of total daily liquid intake (flavored stimulus paired with predigested liquid diet + flavored stimulus paired with

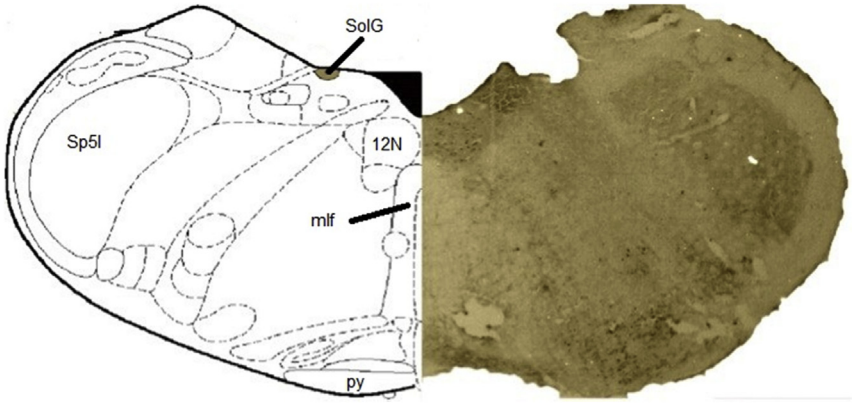

Fig. 1. Histological preparation stained with cresyl violet, showing the localization of the SolG lesion in a representative animal in this experiment (12N: hypoglossal nucleus; Mlf: medial longitudinal fasciculus; Py: pyramidal tract; SolG: nucleus of the solitary tract, gelatinous part; SP5I: spinal trigeminal nucleus, interpolar part).

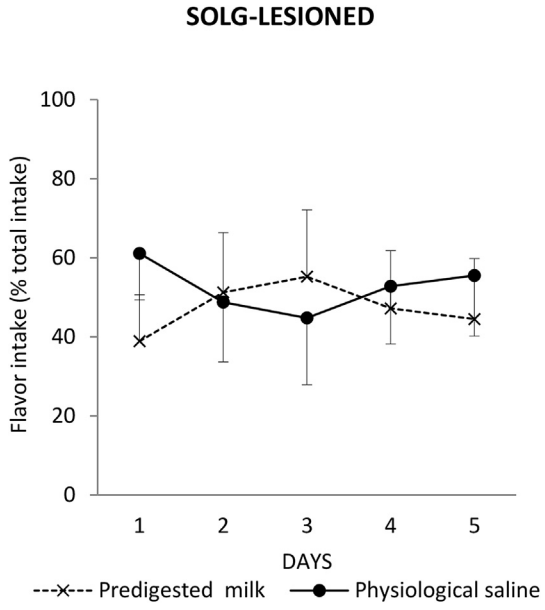

Fig. 2. Mean intakes (% total intake) of flavored stimuli (mL) paired with predigested liquid diet and physiological saline in SolG-lesioned animals.

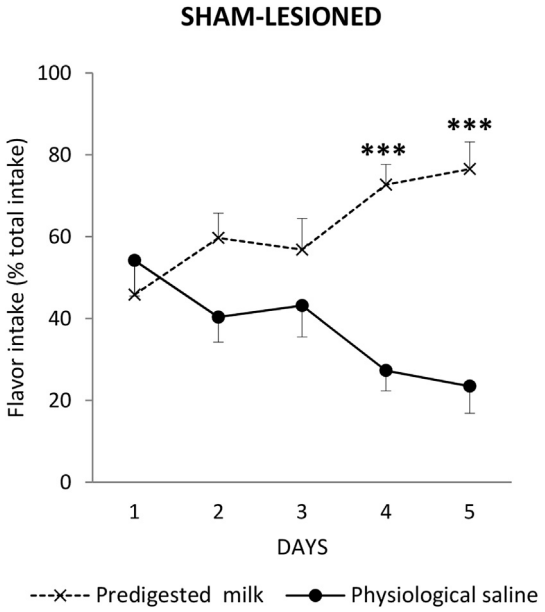

Fig. 3. Mean intakes (% total intake) of flavored stimuli (mL) paired with predigested liquid diet and physiological saline in sham-lesioned animals (***: $p < 0.01$).

physiological saline) between the groups [two-way ANOVA, group x total liquid consumed/day] showed that the effect of group [$F(1,13) = 0.006$, $p < 0.93$] and group x total liquid/day interaction [$F(4,52) = 0.36$, $p < 0.83$] were not significant. However, statistical significance was observed for the effect of total liquid intake over all days [$F(4,52) = 5.63$, $p < 0.0007$; See Fig. 4].

4. Discussion

The results of this study demonstrate that lesions of the SolG subnucleus of the NST disrupt learned concurrent flavor/side preferences induced by intragastric administration of rewarding foods. Intake by the animals of the flavor paired with the PD liquid diet was similar to their intake of the flavor paired with PS on all days of the experiment (Fig. 2). In contrast, this discrimination learning task was correctly accomplished by sham-lesioned animals; thus, although the intake of the two flavors was similar on the first day of the study, these animals progressively opted for the nutrient-associated flavor over the learning period (Fig. 3).

No significant differences were found between the groups in their daily total liquid intake (flavor paired with PD liquid diet + flavor paired with PS). These data suggest that the learning interruption observed in SolG-lesioned subjects was not related to motor, motivational, or other impairments produced by the lesion (this interpretation is supported by the lack of difference in body weight between the groups at any time during the study). These animals appear to be capable of developing other types of learning (i.e., sequential learning) independently of this neural processing axis, as demonstrated after lesions to other anatomic regions from which the SolG receives information (Zafra, Molina et al., 2007) or to which this subnucleus projects (Zafra et al., 2002).

The present study used a concurrent learning procedure (Mediavilla et al., 2005) to test involvement of the SolG in the rapid processing of rewarding information of visceral origin rather than determine characteristics of the learning (i.e., flavor vs. side/place) or possible ongoing factors (measured by extinction tests), which have already been considered in previous studies (Agüera et al., 2016).

It could also be argued that the sham-lesioned animals may have acquired the learning by using delayed long-term (and not

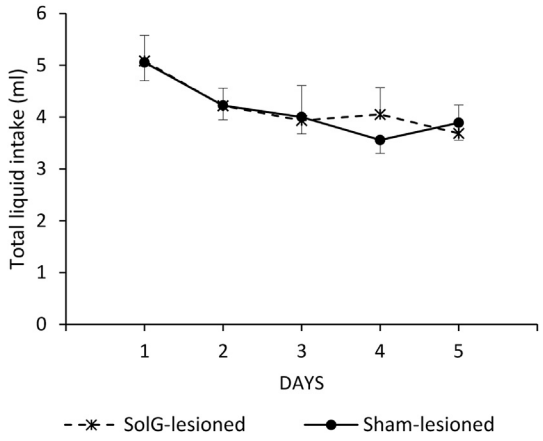

Fig. 4. Mean total liquid intake (flavor paired with predigested liquid diet + flavor paired with physiological saline; mL) of SolG-lesioned and sham-lesioned animals.

short-term) signals, associating the amount of the two flavors consumed with the amount of subsequent nutrient reward (Baker & Booth, 1989). In fact, it has been demonstrated that the gut can monitor the nature and caloric content of intragastrically administered nutrients (Ferreira, Tellez, Ren, Yeckel, & de Araujo, 2012; Tellez et al., 2013). However, if this were the case in the present experiment, animals in the SolG-lesioned group, whose post-adsorptive pathway remained intact, would presumably also have learned the task, which was not observed in the present or previous studies (Zafra, Molina et al., 2007, Zafra et al., 2002). Furthermore, animals were able to establish preferences in a single session in some previous studies (using liquid predigested protein), making the participation of humoral mechanisms unlikely (Puerto, 1977; Zafra, Molina, & Puerto, 2009).

Finally, it should be taken into account that the lesioning technique used in this study means that it cannot be determined whether the consequences of SolG lesion are attributable to damage to cell bodies or to fibers of passage. However, previous research in this line revealed the induction of c-Fos immunoreactivity in NST subnuclei after acquisition of concurrent flavor learning (Mediavilla, Bernal, & Puerto, 2007). In addition, rewarding electrical stimulation of the external lateral parabrachial subnucleus, one of the regions to which the SolG projects, was found to produce contralateral activation of this subnucleus (Simon, 2003). All of these data suggest the involvement, at least partially, of cell bodies.

Similar findings to the present study have been observed after the interruption of neural pathways and brain regions in more caudal and rostral levels than the NST. Thus, disruption of learned concurrent flavor/side preferences was reported after lesions of vagal afferents and after lesions of LPBe (Zafra, Molina et al., 2007, Zafra et al., 2002), a pontine relay structure of the NSTic (Herbert et al., 1990). SolG may therefore be part of a potential rapid gastrointestinal visceral information processing pathway in which the aforementioned visceral-brain components also participate and which may be essential for the induction of concurrent flavor/side preference.

It appears well established that the gut can accurately and rapidly detect luminal nutrient content, which is essential for generating the appropriate motor and secretory responses to a meal (Mei, 1985; Raybould, 2008). For this purpose, cells that express receptors for different macronutrients are contained in the mucosa of both stomach (Depoortere, 2015; Page & Kentish, 2016; Page, Symonds, Peiris, Blackshaw, & Young, 2012) and small intestine (Janssen & Depoortere, 2013; Page & Kentish, 2016; Page et al., 2012; De Lartigue & Diepenbroek, 2016).

This biological substrate of rapid and precise detection is important not only to control the digestive function but also to regulate nutrient preferences. Thus, it has been reported that the perivagal application of capsaicin, a neurotoxin that mainly destroys afferent fibers (Holzer, 1991; Jancsó et al., 1987), interrupts concurrent flavor/side preferences (Zafra, Molina et al., 2007).

Afferent vagal endings do not penetrate the epithelial cell layer or protrude into the lumen and are therefore never directly exposed to gastrointestinal contents. However, they are located in close proximity to the epithelial cells (Berthoud, Kressel, Raybould, & Neuhuber, 1995; Powley, Spaulding, & Haglof, 2011) and appear to receive detailed information on the types of nutrient present in the gastrointestinal tract. This is received both directly, *via* luminal chemicals freely diffused across epithelial cells (vagal afferent neurons express receptors for numerous macro- and micronutrients) and also *via* paracrine messengers released by enteroendocrine cells, which act as sensory transducers ("taste" cells) that monitor the physical and chemical nature of luminal contents (Dockray, 2013; Grundy, 2006; Janssen & Depoortere, 2013; Page & Kentish, 2016; Page et al., 2012; Raybould, 2008; Zhu, Zhu, Owyang, & Li, 2001; De Lartigue & Diepenbroek, 2016).

The well-documented activation of vagal afferents in response to macronutrients (carbohydrates, amino-acids and peptides, and fats) and changes in pH or osmolality (Berthoud et al., 1995; Grundy, 2006; Iggo, 1957; Powley et al., 2011; Raybould et al., 2006; Sengupta & Gebhart, 1994) takes place within a short time period (Blackshaw et al., 1987; Clarke & Davison, 1974; Melone, 1986; Sengupta & Gebhart, 1994; Zhu et al., 2001). The vagus nerve therefore possesses the neurobiological mechanisms required to: (1) detect the presence of food in the gut, *via* mechanosensitive mucosal vagal afferents; (2) obtain precise information on luminal nutritive contents, *via* chemosensitive mucosal vagal afferents; and (3) rapidly communicate this information to the brain (Page & Kentish, 2016; Page et al., 2012; Powley et al., 2011).

Nutrient visceral information is transmitted to the brain through vagal afferents that appear to be concentrated in the NSTic, especially in the medial division of this nucleus (Altschuler et al., 1989; Barraco et al., 1992). Specifically, vagal afferents from the stomach preferentially project to the SolG (Altschuler et al., 1989; Rinaman & Schwartz, 2004; Zhang et al., 1995), which receives both mechanosensitive (Emond, Schwartz, & Moran, 2001; Olson, Freilino, Hoffman, Stricker, Sved, & Verbalis, 1993; Rinaman, Baker, Hoffman, Stricker, & Verbalis, 1998; Sabbatini et al., 2004; Zhang et al., 1995) and chemosensitive (Emond et al., 2001; Olson et al., 1993; Rinaman et al., 1998) gastric information. As noted above, many of the vagal fibers that end in the SolG are capsaicin-sensitive (Jancsó & Király, 1980).

The dorsomedial subnucleus and the SolG it contains project to the LPBe (Herbert et al., 1990), which receives information from the stomach (Rinaman & Schwartz, 2004; Yamamoto & Sawa, 2000) and other gut segments (Wang, Cardin, Martínez, Taché, & Lloyd, 1999) and is also sensitive to electrical stimulation of the vagus nerve (Gieroba & Blessing, 1994; Saleh & Cechetto, 1996) and NSTic (Suemori, Kobashi, & Adachi, 1994). This subnucleus has proven essential to establish concurrent flavor/side preferences induced by intragastric administration of "cephalic" PD nutrients (Zafra et al., 2002), and to establish concurrent place preferences induced by electrical stimulation (Simon, Garcia, Zafra, Molina, & Puerto, 2007; Simon, Zafra, Molina, & Puerto, 2008, Simon, Garcia, & Puerto, 2011).

Given that the SolG is an NST subnucleus that almost exclusively receives gastric vagal afferents (Altschuler et al., 1989; Barraco et al., 1992; Shapiro & Miselis, 1985), the present findings suggest a certain involvement of the stomach in the establishment of food

preferences using the concurrent learning modality. This conclusion is supported by the fact that the liquid diet in the present study contained many of the macronutrients that can be detected in the stomach, including carbohydrates, medium- and long-chain fatty acids, amino-acids, and salts (Depoortere, 2015; Page & Kentish, 2016; Page et al., 2012; Uneyama, Niijima, San Gabriel, & Torii, 2006). However, based on the available data, we are unable to confirm whether or not this is the case or whether other digestive segments (e.g., duodenum) are involved.

Interestingly, gastric epithelial cells also appear to be sensitive to bitter tastants (Janssen & Depoortere, 2013). In this context, the vagus nerve-NSTic-LPBe rapid processing axis has also proven necessary for the transmission of noxious visceral substances; specifically, with some probable anatomical variants, it has been implicated in concurrent taste aversion (Arnedo, Gallo, Agüero, & Puerto, 1990; Zafra, Prados et al., 2006; Mediavilla, Molina, & Puerto, 2000, 2011). In contrast, has been documented that the vagus nerve (Zafra, Prados et al., 2006), NSTic (Mediavilla, Bernal, Mahía, & Puerto, 2011) and LPBe (Mediavilla et al., 2000) are not essential for flavor aversion learning if there is no requirement for the rapid processing of a visceral stimulus, as in learned food preferences (Zafra et al., 2002, Zafra, Molina et al., 2007).

This vagus nerve-NSTic-LPBe axis also appears to be relevant in nausea and vomiting, protective mechanisms to expel potentially harmful substances from the alimentary tract (Boissonade & Davison, 1996). It has been reported that vagotomy can (Babic & Browning, 2014; Boissonade & Davison, 1996; Horn et al., 2014; Reynolds, Barber, Grahame-Smith, & Leslie, 1991) or cannot (Gupta, Schafer, Ramaroson, Sciullo, & Horn, 2017; Horn et al., 2014, Horn, De Jonghe, Matyas, & Norgren, 2009) abolish this mechanism, depending on the type and dose of emetics used. Fos-like or viral immunolabeling techniques have demonstrated that emetic agents transmitted to the brain *via* the vagus nerve are processed by the NST and lateral parabrachial subnuclei (including SolG and LPBe) (Babic & Browning, 2014; Horn et al., 2014; Reynolds et al., 1991), and this processing can also be abolished by vagotomy (Reynolds et al., 1991).

Finally, the rapid processing pathway involved in inducing learned concurrent flavor/side preferences also appears to be important in other behavioral processes requiring the rapid transmission of nutritive visceral information (Zafra, Molina, & Puerto, 2003). Thus, it has recently been reported that the vagus nerve (Zafra, Molina et al., 2016), SolG (Zafra, Agüera, Molina, & Puerto, 2017a), and LPBe (Zafra, Agüera, Simón, Molina, Puerto, 2016) are essential in the re-intake of food after the partial extraction of gastric contents immediately after ending a meal. Neurologically intact animals re-ingest approximately the same amount extracted, while lesioned animals cannot compensate for the deficit created and consume a much smaller amount.

In conclusion, the results of this study suggest that SolG is important in the rapid visceral information processing involved in the induction of learned concurrent flavor/side preferences, in which signals from the stomach might have a preponderant role. This rapid processing pathway, which also includes the vagus nerve and LPBe, would also be relevant in other behavioral processes that require the rapid transmission of information from the upper gastrointestinal tract.

Acknowledgements

The authors are grateful to Richard Davies for assistance with the English version of this paper. Grant Support: This work was supported in part by the University of Granada and Spanish Ministry of Education and Culture (Nacional R+D Plan: PSI2010-17400). Some of these data have been presented in abstract form in the 2nd International Congress of Psychobiology, Avila, Spain (Zafra, Agüera, Molina, & Puerto, 2017b).

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