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Research report

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

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ABSTRACT

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not able to compensate for the deficit created, and their re-intake of food was significantly lower than the amount withdrawn after the satiating meal. In contrast, the food re-intake of control animals was similar to the amount withdrawn.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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

2.1. Subjects

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

2.2. Surgical procedure

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

Surgery was carried out under general anesthesia with sodium pentothal (50 mg/Kg, ip; sodium thiopental, Abbot Laboratories, Spain). Once animals were anesthetized, they were placed in a stereotaxic device (Stoelting Co. Stereotaxic 51.600), and a cathodic electric current (0.3 mA) was bilaterally applied for 10 s using a DCML-5 lesion-maker (Grass Instruments Corp., Quincy, Mass, USA). Electric current was supplied through a 00 stainless steel monopolar electrode, approximately 200 μm in diameter, and insulated throughout its length except for the last 0.5 mm. The anatomical coordinates for the LPBe were obtained (interaural references) from the Paxinos and Watson stereotaxic atlas (Paxinos and Watson, 1996): anterior/posterior (AP) = −0.16 mm; lateral (L) = ± 2.5 mm; and ventral (V) = +3.0 mm.

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

2.2.2. Intragastric catheter

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

2.3. Behavioral procedure

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

2.4. Experiment 1A

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

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

2.5. Experiment 1B

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