Brief report

Capsaicin-sensitive afferent vagal fibers are involved in concurrent taste aversion learning

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Abstract

Taste aversion learning (TAL) is a type of learning characterized by rejection of a gustatory/flavor stimulus as a consequence of its pairing with visceral discomfort and malaise. TAL can be established in the laboratory by two different behavioral procedures, concurrent or sequential. Neural mechanisms of these learning modalities remain to be elucidated, but several studies have discussed the implication of various anatomical structures, including the vagus nerve. The aim of this study was to examine the role of capsaicin-sensitive vagal afferent fibers in concurrent (Experiment 1) and sequential (Experiment 2) TAL in Wistar rats. Results showed that perivagal administration of capsaicin (1 mg of capsaicin dissolved in 1 ml of vehicle (10% Tween 80 in oil)) blocked acquisition of concurrent but not sequential TAL. These data support the hypothesis of two different modalities of TAL mediated by distinct neurobiological systems, with vagal nerve participation only being essential in concurrent TAL.

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The vagus nerve has been implicated in various behavioral processes, including thermoregulation, food and water intake, immune function, nausea and vomiting, and taste aversion learning (TAL). In TAL, animals learn to avoid a flavor previously paired with gastrointestinal malaise. TAL can be established in the laboratory by using two distinct experimental procedures: concurrent or sequential. In the former, two flavored stimuli are presented at the same time, one associated with simultaneous intragastric administration of a noxious product and the other with saline; in the latter, the two flavored stimuli are presented at alternate sessions (Mediavilla, Molina, & Puerto, 2005).

Participation of the vagus nerve in TAL is supported by some studies (Arnedo, Gallo, Agüero, Molina, & Puerto, 1993; Coil, Rogers, García, & Novin, 1978; Fox & McKenna, 1988) but disputed by others (Hunt, Rabin, & Lee, 1987; Kiefer, Rusiniak, García, & Coil, 1981; Martin, Chen, & Novin, 1978). The objective of this study was to specifically examine the involvement of the afferent component of the vagus nerve in TAL. Moreover, since concurrent TAL requires a rapid processing of visceral information (Mediavilla et al., 2005), it can be hypothesized that the integrity of the vagus nerve is especially necessary in this learning modality. Capsaicin was used, a neurotoxin that selectively destroys weakly myelinated afferent fibers (Aδ) or C unmyelinated fibers (Berthoud, Patterson, Willing, Mueller, & Neuhuber, 1997; Hölzer, 1991), both abundant in the vagus nerve (Prechtl & Powley, 1990; Sengupta & Gebhart, 1994). Capsaicin was locally applied on the esophagus in the subdiaphragmatic region, allowing vagal afferents that originate from the thoracic viscera to remain intact. Hypertonic NaCl, known to act on vagal afferents, was used as noxious agent (Mei & Garnier, 1986; Zhu, Wu, Owyang, & Li, 2001).

Previous studies demonstrated that vagal deafferentation with capsaicin induces an increased food intake 24h after the surgery, and this effect has been used in this paper as a behavioral test of the lesioning effect of the perivagal application of capsaicin (Zafra, Molina, & Puerto, 2003).
All experiments were performed on adult male Wistar rats from a breeding colony at the University of Granada (283–392 g). Experiment 1 used 24 rats (capsaicin-treated group: N = 13, control sham-procedure group: N = 11), and Experiment 2 used 20 rats (capsaicin-treated group: N = 10, control sham-procedure group: N = 10). On arrival at the laboratory, the animals were individually housed with unlimited access to food (Panlab, S.L. Barcelona, Spain) and water. The front side of the cage had two 1.6-cm holes at the same distance from the center and edge (left or right) and at the same height above the floor of the cage. The animal had access through these orifices to spouts attached to cylindrical graduated burettes for administration of flavors and water (see Fig. 1 in Mediavilla et al., 2005). The room was maintained on a 12-h light/12-h dark cycle and at 21–23°C. The experimental procedures took place during light periods and were conducted in accordance with the Animal Care and Use Guidelines established by Spanish Royal Law 223/1988.

The perivagal capsaicin treatment was performed following a modification of the method published by Raybould and Taché (1989). Rats were anesthetized with sodium pentothal (46.3 mg/kg, ip; Abbot Laboratories, Madrid, Spain) and a 3-cm-long midline laparotomy incision was made through the abdominal wall. The esophagus was exposed and paraﬁlm lamina was placed beneath it to minimize the spread of capsaicin (Fluka, 98%) to surrounding tissues. A cotton pledget soaked in capsaicin solution (1 mg of capsaicin dissolved in 1 ml of vehicle (10% Tween 80 in olive oil)) was placed around the esophagus for 30 min. Capsaicin drops were applied every 5 min to keep the cotton moist. The total amount of capsaicin applied was 1 ml (1 mg/rat). During the 30-min capsaicin application, two intragastric catheters were implanted into the cardiac portion of the stomach and routed through the abdominal muscle wall, one on each side of the animal, to the back of the neck. The area was then thoroughly rinsed with saline and dried with sterile swabs, and stitching was performed as needed to help close the wounds. A local antiseptic was applied to the wound (Betadine, Viatris Pharm., Madrid, Spain) and subjects received an i.m. 0.1 cm3 dose of penicillin (1,000,000 IU, Penilevel. Lab. Ern, Barcelona, Spain). The surgical procedure for the control animals was identical except that only the vehicle for capsaicin (10% Tween 80 in olive oil) was perivagally applied.

A capsaicinization behavior index was obtained by recording the amount of food consumed during the day before the surgical intervention and at 24, 48, and 72 h after the end of surgery. In order to test whether the vagus nerve accidentally suffered complete damage during the surgical intervention, the animals underwent a complete vagotomy test after the experiments, using the Martin et al. (1978) procedure.

After 7–8 days of postoperative recovery, subjects underwent a 3-day pre-training period of water deprivation. During these 3 days, animals in Experiment 1 were allowed to drink tap water from two graduated burettes offered simultaneously. During pre-training days 1 and 2 of Experiment 2, animals had access to only one burette containing tap water and its position was varied appropriately to avoid development of positional preferences, and on day 3 both burettes were offered simultaneously. In both experiments, the water was offered for 10 min on day 1 of the pre-training period and for only 7 min on days 2 and 3. Thirty minutes after removal of the water, the animals were provided with 15 g of food. Three animals from Experiment 1 were excluded during this phase, two from the lesioned group and one from the control group, because one of their catheters became detached.

Experiment 1 aimed to examine the participation of capsaicin-sensitive vagal fibers in concurrent TAL. This 3-day experiment began the day after the pre-training period. In each experimental session, the rats were given the choice of two simultaneously offered gustatory stimuli for 7 min (0.5% strawberry [S] and 0.5% coconut [C], McCormick Co. Inc., San Francisco, CA). Intake from one 0.1-cm3-graduated burette was paired with simultaneous intragastric injection of an aversive substance (hypertonic NaCl, 5%), whereas intake from the other burette was paired with simultaneous intragastric injection of physiological saline (PS, Apiroserum Lab. YBIS, Madrid, Spain). In order to control for any flavor preferences, the flavor paired with the aversive substance (5% NaCl) was counterbalanced across animals.

Experiment 2 aimed to examine the implication of capsaicin-sensitive vagal fibers in sequential TAL. The experimental learning test (3 days) commenced the day after the 3-day pre-training period. On day 1, animals were offered a

**Fig. 1. Mean food intake by the capsaicin-treated and control groups in experiments 1 (A) and 2 (B) at 24, 48, and 72 h after the surgical intervention (*p < .05; **p < .01).**
graduated burette containing a gustatory stimulus (0.5% strawberry) through the left orifice of the cage for 7 min. Flavor intake was paired with the simultaneous intragastric injection of 5% NaCl in half the capsaicin-treated and control animals and of physiological saline in the other half. On day 2, animals were offered a burette containing a differently flavored solution (0.5% coconut) through the right orifice for 7 min. Animals receiving NaCl associated with ingestion of strawberry solution on day 1 now received simultaneous intragastric administration of physiological saline, and animals previously administered with physiological saline now received NaCl. On day 3, a choice test was performed in which both gustatory stimuli (S and C) were simultaneously presented for 7 min, each in its respective position. On this test day, the gustatory stimuli were offered without intragastric injections. Throughout the experiments, NaCl and saline were injected at a rate of 1 ml/1 ml of ingested flavor stimulus every time the rats drank from the associated burette. On each day, experimental and control subjects were provided with 15 g of food at 60 min after the drinking period.

Vagotomy tests showed that one control subject in Experiment 1 and one capsaicin-treated animal in Experiment 2 had suffered a complete vagotomy, and they were excluded from the statistical analysis.

There were no statistically significant differences between capsaicin-treated and control animals in food intake during the day before surgery in either Experiment 1 or 2 [all \( p \) values > .05]. After the surgery, capsaicinized rats consumed significantly more food than vehicle-control rats at 24 h \( [F(1,18)=8.36; \ p < .009; \text{one-way ANOVA}] \) but not at 48 or 72 h [all \( p \) values > .05] in Experiment 1 (Fig. 1A), and consumed significantly more than vehicle-control rats at 24 h \( [F(1,17)=6.65; \ p < .019] \) and at 48 h \( [F(1,17)=6.57; \ p < .02] \) but not at 72 h in Experiment 2 [\( p > .05, \text{Fig. 1B} \)].

Analysis of the concurrent TAL data of Experiment 1 using a three-way ANOVA revealed a significant interaction (group \( \times \) day \( \times \) substance) \( [F(2,36)=3.84; \ p < .03] \). Planned intergroup comparisons showed no difference between groups on day 1 or day 2 of the experiment [all \( p \) values > .05] and a significant difference on day 3 [\( F(1,18)=5.83; \ p < .026 \)]. Intragrup comparisons showed no significant differences on any day with the exception of the control group on day 3, when these animals consumed a larger amount of the gustatory stimulus paired with physiological saline \( [F(1,8)=5.35; \ p < .032] \) (see Fig. 2).

Data from the choice test of Experiment 2 were analyzed by means of a two-way ANOVA (group \( \times \) substance). This analysis showed that there were no differences between the groups (Fig. 3); neither interaction nor group effect were significant (all \( p \) values > .05). In contrast, the substance effect was significant \( [F(1,17)=173.009; \ p < .000001] \), with capsaicin-treated \( [F(1,17)=85.2; \ p < .000001] \) and control \( [F(1,17)=88.01; \ p < .000001] \) animals both showing a significantly higher intake of the gustatory stimulus associated with saline.

Results obtained demonstrate that the lesion of capsaicin-sensitive afferent vagal fibers (behaviorally confirmed, Fig. 1; Zafra et al., 2003) blocks concurrent TAL when the aversive visceral stimulation is induced by intragastric administration of hypertonic NaCl. In contrast, the same lesions appear to have no effect on sequential TAL. In the latter case, capsaicin-treated animals performed the task with the same efficacy as the intact animals and avoided intake of the gustatory stimulus associated with the aversive product.

Moreover, the results of Experiment 1 cannot be explained by a learning failure induced by lesion of vagal afferents, since animals with identical neurological damage were able to perform the task in the sequential paradigm.
A fundamental difference between these learning modalities is that the rapid detection of the visceral stimulus is necessary for concurrent TAL, providing the only possibility of associating it with the corresponding gustatory stimulus; in sequential TAL, there is no such time requisite and the visceral stimulus can be processed in capsaicinized animals by the humoral pathway (Carlson & Osborn, 1998; Mediavilla et al., 2005; Starbuck, Wilson, & Fitts, 2002), which is not effective in concurrent learning.

The noxious substance selected for our study (NaCl) meets the necessary requirements for the development of concurrent TAL (Blackshaw & Grundy, 1993; Mei & Garnier, 1986). These requirements are not met, for example, with the use of LiCl, which produces vagal stimulation at around 10 min after its administration (Niijima & Yamamoto, 1994).

These data add to and extend previous findings by our laboratory (Arnedo et al., 1993), specifically implicating capsaicin-sensitive slightly myelinated afferent fibers (Aδ) or unmyelinated C nerve fibers in concurrent TAL.

Although the noxious effect induced by visceral administration of hypertonic NaCl could be interpreted in terms of pain, previous studies in our laboratory have shown that capsaicin-injured vagal afferents may transmit satiation, for example, but do not process pain information (Zafra, Molina, & Puerto, 2004). This suggests that the visceral sensation induced is not nociceptive but is probably aversive.

In conclusion, these data support the existence of a dual visceral-cerebral system in TAL, as observed in nutrition, emesis, and immune systems (Mediavilla et al., 2005). Specifically, the vague nerve, the afferent of capsaicin-sensitive vagal fibers, participates in concurrent but not sequential TAL.

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References


