Conditioned Taste Aversion Induced by Motion Is Prevented by Selective Vagotomy in the Rat

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The role of the vagus nerve in motion-induced conditioned taste aversion (CTA) was studied in hooded rats. Animals with complete, selective gastric vagotomy failed to form conditioned taste aversion after multiple conditioning sessions in which the conditioned stimulus (a cider vinegar solution) was drunk immediately before a 30-min exposure to vertical axis rotation at 150°/s. Results are discussed with reference to the use of CTA as a measure of motion-induced "sickness" or gastrointestinal disturbance, and, because motion-induced CTA requires that both the vagus nerve and the vestibular apparatus be intact, in light of the possible convergence of vagal and vestibular functions. © 1988 Academic Press, Inc.

The avoidance of a previously novel food which has been ingested just prior to toxicosis or irradiation is a well-documented form of associative learning called conditioned taste aversion (CTA). This learned aversion is considered to result from a form of classical conditioning in which the novel food serves as a conditioned stimulus (CS) that is followed by (i.e., paired with) an unconditioned stimulus (US), the toxicosis or irradiation.

It has been suggested that CTA might be used as a species-specific measure of motion sickness in animals which do not vomit (Mitchell, Krusemark, & Hafner, 1977) or as a measure of prodromal symptoms of motion sickness (i.e., nausea) in species which do vomit (Roy & Brizzee, 1979). These suggestions assume that motion-induced CTA results

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from some form of general malaise or aversive internal state produced by the motion. If CTA and the emetic reflex are to be considered alternative measures of motion sickness, then it is expected that they should share common neural pathways. While important neural pathways have been identified for both the emetic reflex arc and CTA, few experiments have directly examined the neural routes important to motion-induced CTA.

One neural pathway which is known to be important in CTA and the emetic reflex involves the area postrema (AP). The AP is a circumventricular site where there is a relatively rapid exchange of substances between the blood and interstitial fluid (Borison, 1974) and it serves as a chemoreceptive site for the emetic action of several toxins (Borison, 1974; Borison & Wang, 1953). Some blood-borne toxins are ineffective USs for inducing CTA when the AP is destroyed in rats. Ablation of AP either eliminates or attenuates the efficacy of scopolamine methyl nitrate (Berger, Wise, & Stein, 1973), lithium chloride (Ritter, McGlone, & Kelly, 1980), intravenous copper sulfate (Coil & Norgren, 1981), and γ radiation (Ossenkopp & Giugno, 1985) as USs. Thus, with certain toxins, the data are consistent with the expectation that CTA and the emetic reflex might share a common neural pathway.

The AP was long thought to be involved critically in vomiting induced by motion (Wang & Chinn, 1954). But recent studies have caused a reexamination of this question (Borison & Borison, 1986; Corcoran, Fox, Brizzee, Crampton, & Daunton, 1985; Wilpizeski, Lowry, & Goldman, 1986), and it is unlikely that AP plays an indispensable role in motion sickness. When motion is the US for inducing CTA in rats, ablation of AP either does not affect (Sutton, Fox, & Daunton, 1988) or enhances (Ossenkopp, 1983) CTA. Motion is an effective US for CTA when AP is destroyed in cats (Corcoran et al., 1985) and squirrel monkeys (Elfar, Brizzee, Fox, Corcoran, Daunton, & Coleman, 1986; Wilpizeski & Lowry, 1987).

A second neural pathway which might be important in both CTA and vomiting involves the vagus nerve. Gastric motility decreases (Schwab, 1954) and tachygastria occurs (Stern, Koch, Leibowitz, Lindblad, Shubert, & Stewart, 1985) during the development of motion sickness in humans indicating vagal afferents could contribute to the total complex of symptoms associated with motion sickness. With regard to CTA, toxins which produce pica, the consumption of nonnutritive substances, and anorexia in rats also can produce CTA (Mitchell, Wells, Hoch, Lind, Woods, & Mitchell, 1976). The observation that pica is reported to occur in humans suffering from gastrointestinal malaise is consistent with the assertion that vagal afferent activity may contribute to CTA produced by motion (Mitchell, Laycock, & Stevens, 1977) or gastric irritants such as intragastric copper sulfate (Coil, Rogers, Garcia, & Novin, 1978). A precise function has not been identified for vagal afferents in CTA produced with copper
sulfate as a US. Vagotomy has been reported to disrupt (Coil et al., 1978) and to enhance (Rabin, Hunt, & Lee, 1985) CTA in rats when copper sulfate is the US. Vagal afferents do appear to influence CTA induced by the effects of copper sulfate on the gut; thus, if gastrointestinal effects occur in rats during rotation, vagal afferents could be involved in the development of CTA when motion is used as an US. This experiment was conducted to determine whether vagotomy affects the formation of CTA when motion is the US.

METHODS

Subjects

A total of 30 Long-Evans rats purchased from Simonsen Laboratories in Gilroy California were used in the experiment. The animals were housed individually in suspended wire-mesh cages with Wayne Rodent Blox available at all times during the experiment. Water was restricted during conditioning as described below. The colony room was maintained on a 12:12 h light:dark cycle with the light period commencing at 7:00 AM. Conditioning was conducted between 1:00 and 3:00 PM during the light phase of the light:dark cycle.

Animals were assigned to the three experimental conditions by a random procedure so that nine rats were in the Intact and Ligation Groups and 12 rats were in the Vagotomy Group. The Ligation Group was used to control for reduced gastric blood supply which occurred in animals in the Vagotomy Group as an outcome of the surgical procedure described below.

Procedure

Surgery. Vagotomies were performed using an adaptation of the method described by Martin, Rogers, Novin, and Vanderweele (1977). The animals were anesthetized with a mixture of 1.50 ml ketamine-HCl (Vetalar, 100 mg/ml), 0.75 ml xylazine (Rompun, 20 mg/ml), 0.30 ml Acepromazine maleate, and 0.45 ml isotonic saline administered intraperitoneally (1 ml/kg). The stomach was exposed with a midline incision extending 2.5 cm from the xiphoid process toward the umbilicus. The stomach was retracted to expose the esophagogastric junction, and the anterior trunk of the vagus was dissected and sectioned distal to the hepatic branch. The stomach was then rotated to expose the posterior trunk of the vagus. The posterior vagus nerve and the gastric artery were identified in the fatty mesentery close to the esophagogastriic junction. Two 2-0 silk sutures were tied around the gastric artery and the nerve 1–2 cm apart and the artery and the nerve were sectioned between sutures. Gastric bundles in the region of the cardiac sphincter and along the greater curvature of the stomach were identified by staining with methylene blue, dissected,
and cut with ophthalmic scissors. After the vagus fibers were sectioned the muscle layers were closed with a continuous suture using 2-0 gut, and the skin was closed with interrupted 2-0 silk sutures. The gastric arterial ligation procedure consisted of similar operative procedures, including tying two silk sutures around the gastric artery and the posterior vagus nerve and staining of anterior and posterior nerve branches. However, no nerves were sectioned during this ligation procedure. Some damage may have occurred to the posterior branch of the vagus due to crushing of the nerve by ligation, but the anterior branch of the vagus should not have been damaged.

**Verification of Vagotomy.** The completeness of vagotomy was verified using two methods discussed by Louis-Sylvestre (1983): the loss of body weight early after surgery and a measure of gastric stasis. Gastric stasis was assessed by excessive retention of food following a fast. After conditioning tests were completed animals were returned to ad lib food and water for 48 h and then fasted for 15 h preceding sacrifice. Under surgical anesthesia stomach contents were removed and weighed. Euthanasia was then induced with sodium pentobarbital (100 mg/kg intramuscularly). Each animal with more than 1.0 g of food retained after fasting and who had lost more than 10% of body weight within 6 days following surgery (Clarkson, King, Hemmer, Olson, Kastin, & Olson, 1982) was judged to have a complete vagotomy.

**Drinking regimens.** Animals were adapted to a restricted drinking schedule prior to surgery by gradually reducing the duration of daily access to water from continuous access to 12 h (3 days), then 6 h (2 days), and finally to 2 h per day (5 days). This procedure facilitated rapid adaptation to a similar schedule after recovery from surgery so conditioning could be conducted before vagal nerve fibers could regenerate (Powley, Prechtl, Fox, & Berthoud, 1983).

For the daily drinking regimen used during conditioning the animals were permitted an initial drinking opportunity with a duration of 10 min followed 100 min later by a second session with a duration of 10 min. Thus, the animals were deprived for 22 h and were then permitted two 10-min drinking opportunities, one at the beginning and one at the end of a 2-h period. A one-bottle conditioning procedure as was used in this experiment may severely reduce the fluid intake if animals develop CTA to the solution used as the CS. Because rats tend to eat during and after access to fluid, allowing two daily drinking periods during conditioning facilitated more normal hydration and feeding.

Following adaptation to the preliminary drinking regimen water was available ad lib for 3 days to reestablish normal hydration and eating patterns and to ensure homeostasis prior to surgery. Food was withheld for 12 h preceding surgery as a general precaution against aspiration during anesthesia and surgery.
Surgeries were performed on 2 days. Eight animals (six vagotomy and two gastric ligation) were in Surgery Group I on the first day and 10 animals (three vagotomy and seven gastric ligation) were in Surgery Group II completed 2 days later. After surgery the animals were maintained on ad lib food and water for either 7 days (Surgery Group I) or 5 days (Surgery Group II). Moistened, powdered chow was provided for the first 2 of these days following surgery. Over the next 7 days the animals were readapted to the restricted drinking regimen by reducing the daily access to water for 12 h (1 day), then to 6 h (1 day), and then to 2 h per day (5 days). During the following 7 days of the conditioning period access to water occurred in two 10-min drinking sessions.

Conditioning. A one-bottle conditioning procedure was used. On conditioning days animals were permitted access to a novel flavored liquid (4% (v/v) solution of Heinz vinegar) during the first 10-min drinking period. The amount of fluid consumed by each animal during this period was determined by weighing drinking bottles before and after the drinking session. Conditioning procedures occurred in three sessions on alternate days during a 5-day sequence. An additional test day occurred on the seventh day when the animals were provided access to the vinegar solution but did not experience the US. In each conditioning session the animals were exposed to vertical axis rotation at 150°/s for 30 min beginning 5 min after the end of the first drinking period. At the end of the 30-min rotation period each animal was returned to its home cage. The second drinking period (tap water only) began 100 min after the first ended. Conditioning was initiated 12 to 14 days following surgery to minimize any effects of reinnervation following vagotomy (Powley et al., 1983).

RESULTS

Measures of Vagotomy

All animals subjected to the vagotomy procedure lost more than 10% of their presurgical body weight within 6 days after surgery. According to the stasis measure, vagotomy was judged to be less than complete in two animals. Both of these animals retained less than 1.0 g of food following the 15-h fast and thus were not used in further analyses. Summary statistics for the two measures used to assess the extent of vagotomy are shown in Table I. These data describe the measures for the 27 animals (nine per group) used in all further analyses. Weight loss was greater in both groups subjected to surgery than in the Intact Control Group which was deprived in the same manner but did not undergo any operative procedure. \( t^s (16) > 4.65, p < .01. \) The mean percentage of body weight lost after surgery was greater for the Vagotomy Group than for the Ligation Group, \( t(16) = 2.69, p < .02. \) However, this measure did not effectively identify animals with complete vagotomy because some animals
TABLE I
Descriptive Statistics for the Two Measures Used to Assess the Completeness of Vagotomy

<table>
<thead>
<tr>
<th>Measure</th>
<th>Statistic</th>
<th>Vagotomy</th>
<th>Ligation</th>
<th>Intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Change</td>
<td>Range</td>
<td>-27 to -18</td>
<td>-27 to -6</td>
<td>-12 to +2</td>
</tr>
<tr>
<td>Change (percentage)</td>
<td>Range</td>
<td>2.6 to 7.7</td>
<td>0.04 to 0.43</td>
<td>0.09 to 0.90</td>
</tr>
<tr>
<td>Stomach Contents (grams)</td>
<td>Range</td>
<td>4.6</td>
<td>0.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

subjected to ligation of the gastric artery alone lost a percentage of weight as great as that lost by animals subjected to vagotomy. On the other hand, animals subjected to ligation of the gastric artery retained sufficient stomach stasis so that stomach content retention by these animals did not differ from that of the Intact Group, $t(16) = 1.48, p > .10$.

Conditioning

The mean daily intake of the vinegar solution by animals in each of the three groups is shown in Fig. 1. The mean intake on Experimental Day 1 reflects the amount of vinegar solution drunk before conditioning and thus serves as a baseline measurement for intake. To determine

![Fig. 1. Mean intake of vinegar-flavored water by the three groups in the four drinking sessions. On each experimental day flavored water was drunk immediately before 30 min of rotation. Thus, intake on Day 1 is a baseline preceding conditioning, and the intake on each of the remaining experimental days reflects conditioning effects of rotation from the preceding experimental day.](image-url)
whether the mean intake by the three groups differed before conditioning, a randomized one-way analysis of variance was conducted on the intake data from this baseline measurement. No reliable differences between groups were reflected by this analysis, \(F(2, 24) < 1.0\). A 3 (groups) \(\times\) 4 (days) mixed analysis of variance with repeated measures on the second factor was used to conduct an overall analysis of the intake data. Both the main effect for Days, \(F(3, 72) = 28.50, p < .001\), and the interaction of Groups with Days, \(F(6, 72) = 5.10, p < .001\), were statistically reliable. Intake by the Intact and Ligation Groups decreased with successive days (Experimental Days 2, 3, and 4) reflecting the development of CTA. For both groups the mean intake on Days 3 and 4 was less than that on Day 1, \(t's(8) > 3.75, p < .01\). In contrast, the mean intake of vinegar solution by the Vagotomy Group did not vary over the Experimental Days \(t's(8) < 0.76, p > .50\) indicating CTA did not develop in animals with complete vagotomy.

**DISCUSSION**

The principle finding of this study is that repeated exposure to rotation failed to produce CTA in rats subjected to vagotomy. This finding suggests that vagus nerve activity contributes importantly to the conditioning effects of rotation as an US.

Neural damage from ligation was not assessed in this experiment, but the crushing effect of ligation certainly must have disrupted axonal transport, and may have induced alterations in protein synthesis in the cell bodies of the posterior branch of the vagus nerve (Dahlin, Nordborg, & Lundborg, 1987). The observation that rats subjected to this ligation procedure formed CTA in a manner that was indistinguishable from that of intact rats suggests that the posterior branch of the vagus is not required for motion-induced CTA, and, therefore, implies that the ventral branch, the sympathetic branch, or both are required.

Reports of the effects of vagotomy on CTA induced with intragastric copper sulfate as an US have produced conflicting results regarding the role of visceral afferents in CTA. Coi et al. (1978) reported that vagotomy prevented CTA but Rabin et al. (1985) found the magnitude of CTA enhanced in rats subjected to vagotomy. Rabin et al. argued procedural differences would not account for these conflicting findings, but the precise cause of the differences is unknown. Significant differences in the outcome of vagotomy could result from variability in the organization of either subdiaphragmatic vagal nerve fibers or paraganglia, or from differences in regeneration of fibers following surgery. In both of the previous studies a recovery period of 4 to 6 weeks occurred between surgery and conditioning. In the present study this recovery period was reduced to 12 to 14 days in order to minimize variability produced by the possible regeneration of fibers (Powley et al., 1983).
The disruption of CTA by vagotomy shown here is consistent with the concept that afferent fibers of the vagus signal gastrointestinal disruption which serves as the proximal US for the development of motion-induced CTA. However, a precise role for visceral innervation in this capacity cannot be determined from this experiment. The surgical procedure used here interrupted both efferent and afferent fibers from the stomach, thereby eliminating vagovagal gastric functions. This procedure probably did eliminate gastric sensory input but it also eliminated the effector functions of the vagus, and it is not clear whether this disruption of the vagovagal circuitry impacts CNS functions which are important to CTA. The disruption of CTA reported here could result from the disturbance of normal functions in unidentified neural networks in the CNS or PNS where vagovagal and other neural inputs normally converge. Convergence of vagal and vestibular functions is implied indirectly because motion-induced CTA is attenuated by the disruption of labyrinthine function (Haroutunian, Riccio, & Gans, 1976; Hartley, 1977; also discussed in Ashe & Nachman, 1980). Thus, it appears that motion-induced CTA requires both labyrinthine and vagal functions. Convergence of vagal and vestibular circuitry is further indicated by the fact that the rate of afferent discharge in the vagus nerve is reduced by caloric stimulation of the labyrinth (Nijjima, Jiang, Daunton, & Fox, 1987). The important brain areas which may be altered by sectioning the vagus nerve cannot be specified with certainty, but the AP, periaqueductal gray matter, nucleus tractus solitarius, and amygdalar complex are areas which are known to be important to CTA and to have primary or secondary interconnections with vagal afferents (Ashe & Nachman, 1980). In the absence of a clear knowledge of vagovagal interactions with various brainstem and/or higher CNS functions, the attribution of the effects shown here to sensory functions alone would be premature.

REFERENCES


VAGOTOMY AND CTA


