BEHAVIORAL AND NEURAL BIOLOGY 50, 133–152 (1988)

Role of the Area Postrema in Three Putative Measures of Motion Sickness in the Rat

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After thermal cauterization of the area postrema in rats the absence of conditioned taste aversion to sucrose paired with lithium chloride (0.15 *M*, 3.3 ml/kg) was used as a pharmacologic/behavioral index of area postrema damage. In a subsequent experiment the effects of area postrema lesions on three measures proposed as species-relevant measures of motion sickness were studied, using off-vertical rotation at 150°/s for either 30 or 90 min. Lesions of area postrema did not alter postrotational suppression of drinking or amount of defecation during motion. The initial acquisition of conditioned taste aversion to a novel cider vinegar solution paired with motion was not affected by lesioning of the area postrema, but these taste aversions extinguished more slowly in lesioned rats than in sham-operates or intact controls. Results are discussed in terms of proposed humoral factors which may induce motion sickness and in light of recent data on the role of the area postrema in similar measures in species possessing the complete emetic reflex. © 1988 Academic Press, Inc.

Conditioned taste aversion (CTA) to novel-tasting foods paired with toxicosis is a well-documented behavioral paradigm which seems related to the natural tendency of animals to avoid ingestion of toxic substances (Barker, Best, & Domjan, 1977; Garcia, Hankins, & Rusniak, 1974). Research on the underlying physiological mechanisms of CTA suggests that drug-induced CTA can be mediated by at least three neural pathways. For example, aversions resulting from gastrointestinal irritation caused by copper sulfate apparently depend on vagal afferents (Coil, Rogers, Garcia, & Novin, 1978; but, see Rabin, Hunt, & Lee, 1985) and those produced by blood-borne toxins such as lithium chloride (LiCl) depend on the area postrema (AP) (Ritter, McGlone, & Kelley, 1980). The integrity

[1] Supported by San Jose State University Foundation Research Grant 09-01-6800.76 and by Cooperative Agreement NCC 2-OR675-801 between NASA–Ames Research Center and San Jose State University. A preliminary report of the data was presented at the 13th Meeting of the Society for Neuroscience, Boston, MA, 1983. We thank Dr. D. A. Hovda for assisting with the statistical analyses. The experiments were conducted at Ames Research Center and conformed to the Center's requirements for the care and use of animals. Send requests for reprints to Dr. Fox.

0163-1047/88 $3.00

of the AP is not a necessary condition for the formation of CTA induced by some nontoxic unconditioned stimuli (US), such as amphetamine (Berger, Wise, & Stein, 1973; Rabin, Hunt, & Lee, 1987; Ritter et al., 1980). However, amphetamine-induced CTA is prevented by lesions of the dorsolateral tegmentum (Wellman, McIntosh, & Guidi, 1981).

Lesioning of the AP, a circumventricular organ located on the floor of the fourth ventricle, has implicated this structure in mediation of the emetic response to drugs (Borison, 1974; Borison & Wang, 1953) as well as to X-irradiation (Brizzee, Neal, & Williams, 1955; Wang, Renzi, & Chinn, 1958). In addition, the AP has been proposed to be a critical structure in the motion sickness reflex arc (Brizzee, Ordy, & Mehler, 1980; Wang & Chinn, 1954). Studies in the rat have shown that AP lesions attenuate or abolish CTA induced by many drugs including LiCl and methylscopolamine (Berger et al., 1973; McGlone, Ritter, & Kelley, 1980; Ossenkopp, 1983; Ritter et al., 1980) as well as CTA caused by X-irradiation (Ossenkopp & Giugno, 1985; Rabin, Hunt, & Lee, 1983). Since the rat is incapable of vomiting (Hatcher, 1924) it has been suggested that CTA produced by rotational stimulation in the rat (Braun & McIntosh, 1973) may be a species-specific manifestation of motion sickness (Mitchell, Krusemark, & Hafner, 1977). This proposal that CTA in nonemetic species may reflect motion sickness seems feasible since whole-body motion produces CTA to novel food in the squirrel monkey (Roy & Brizzee, 1979). If CTA induced by motion is to be considered a measure of motion sickness, then it is expected that common neural pathways should mediate both CTA and the emetic reflex. However, contrary to expectations from studies on the role of the AP in dog (Wang & Chinn, 1954) and squirrel monkey (Brizzee et al., 1980), Ossenkopp (1983) found that lesioning of the AP in rat *enhanced* rather than prevented development of motion-induced CTA.

Haroutunian, Riccio, and Gans (1976) proposed that the suppression of drinking following rotation is another useful index of motion sickness in the rat. These authors reported that the degree of suppression of postrotational intake of water by thirsty rats was directly related to the duration of treatment, consistent with the finding that the magnitude of motion-induced CTA increases with longer periods of rotation (Green & Rachlin, 1976). Thus, both motion-induced CTA and suppression of drinking are sensitive to the magnitude (or dose) of rotation, in a manner similar to the dose-dependent effects reported for drug-induced CTAs (Nachman & Ashe, 1973; Rabin et al., 1987; Rauschenberger, 1979).

The importance of defecation as a symptom of motion sickness in man (Money, 1970) has led to its inclusion into scales rating the severity of motion sickness in cat (Suri, Crampton, & Daunton, 1979) and monkey (Igarashi, Isago, O-Uchi, Kulecz, Homick, & Reschke, 1983). Ossenkopp and Frisken (1982) reported that rats subjected to motion exhibit significant

increases in defecation during motion compared to sham-rotated rats and concluded that defecation was a species-relevant indicator of motion sickness in the rat.

The experiments reported here were conducted to investigate further the role of the AP in the formation of motion-induced CTA and to evaluate the usefulness of defecation and suppression of drinking as measures of motion sickness. Before initiating conditioning procedures using motion as the US, conditioning using LiCl as the US was conducted in order to identify animals with effective lesions of the AP and to facilitate the assignment of animals to motion conditions. Both "moderate" (30 min) and "severe" (90 min) motion conditions were used in the experiment. Although it has been shown that the magnitude of CTA and the degree of suppression of drinking are directly related to the duration or severity of rotation, most studies on motion-induced CTA have used only severe motion conditions [cf. Ossenkopp (1983) who reported that ablation of the AP did not block CTA]. The duration of motion was varied in this experiment to investigate whether the role of the AP in motion-induced CTA depends upon the intensity of the US as is the case with drug-induced CTA, which is mediated in a dose-dependent manner with high, but not low, doses of LiCl (Rauschenberger, 1979) and amphetamine (Rabin et al., 1987) inducing CTA even after complete ablation of the AP.

METHODS

Subjects

A total of 120 hooded rats of the Long–Evans strain were used in the experiments. Animals were housed in individual wire mesh cages (18 × 25 × 20 cm) on a 12:12 h light:dark schedule with lights on at 0700. Both food and water were available ad libitum until conditioning procedures were initiated.

Apparatus and Materials

The rotation apparatus consisted of a holding cage on an aluminum disk mounted on a gear reduction box driven by a variable-speed motor. To produce off-vertical rotation, the aluminum platform was tilted 20° from earth vertical and was rotated at 150°/s. The duration of rotation was for 30 min in the "moderate" condition and 90 min in the "severe" rotation condition. A holding cage bolted to the rotation platform was constructed of clear Plexiglas and contained five tiers of four compartments, each measuring 18 × 19 × 14 cm. The bottom of each compartment was fitted with hardware cloth which contained grids large enough to allow fecal boli to fall to the floor of the compartment. Each animal was unrestrained within a compartment and was able to orient toward or away from the axis of rotation. Thus, depending on body orientation, a

centrifugal force of up to 0.16*g* could be present at the head of an animal during rotation. A similar compartmentalized box made of Plexiglas was used for confining animals for no-motion control conditions.

The flavored solutions used in the experiments were 10% (w/v) sucrose and a 4% (v/v) cider vinegar solution (Heinz; pH = 3.75). Solutions were provided to animals in standard water bottles fitted with rubber stoppers holding stainless steel drinking tubes which contained steel balls to minimize leakage. The amount of fluid consumed by each animal during all drinking periods was determined by weighing the water bottles before and after each period of drinking.

Procedures

Surgery. Animals were 132 to 134 days old at the time surgery was performed and were randomly assigned so that 25% were intact controls, 25% were sham-operated controls, and 50% were subjected to lesion of the AP. The sham procedures and AP lesions were performed while animals were anesthetized by a 1 ml/kg im injection of a mixture of Ketamine (50%), Rompun (25%), Acepromazine (10%), and physiological saline (15%). After mounting animals in a stereotaxic holder with the head in a ventroflexed position the occipital bone was exposed and the foramen magnum was carefully enlarged with a rongeur instrument. The area of the obex was visualized with a dissecting microscope (Zeiss, Model 30-06-02) and the posterior medullary velum was cut to allow cerebrospinal fluid to escape from the fourth ventricle. The cerebellum was gently lifted rostrally to allow access to the AP. A loop-tip cautery (Accu-Temp, Concept, Inc., Model 4400) formed to the shape and size of the AP was used to make thermal ablations. The neck muscles and scalp were then sutured closed. Sham-lesioned control animals were subjected to the same surgical procedures but the AP was not cauterized. Of the 90 rats in the lesion and sham control groups, two animals died shortly after surgery and three were euthanized in the postsurgical recovery period when they showed signs of neurological pathology reflecting brainstem damage. Thus, at the time conditioning procedures began there were 30 intact controls, 28 sham controls, and 57 lesioned animals.

Animals were weighed once every third day over a 34-day period of recovery to determine the effects of AP lesions and/or surgery on body weight before initiating conditioning procedures. At the end of this postsurgical recovery period animals were randomly assigned to one of nine groups formed by the factorial combination of three motion conditions and three lesion conditions.

Drinking schedules. Access to water was limited to 20 min per day during conditioning procedures, with two 10-min drinking periods used in each experiment. All animals were adapted to a restricted drinking schedule which allowed 10 min of access to tap water in the home cage

every 24 h for 6 days. Additionally, one half of the animals received 10 min of access to water in the home cage 1 h after the first drinking period and the other animals received a second 10-min access to tap water 2 h after their first daily access period. This second period of access to water was provided to ensure that animals were adequately hydrated and to allow for measures of drinking suppression after conditioning with rotation. The second daily drinking periods were scheduled to allow 30 min for transferring animals to and from the rotation apparatus and the home cages on the rotation conditioning day.

Conditioning with LiCl. After 6 days of adaptation to the restricted drinking schedule all animals were given access to a novel-tasting 10% sucrose solution during the first 10-min access period on Day 7 (LiCl conditioning day). Immediately following removal of this sucrose solution the animals were injected with 0.15 *M* LiCl (3.3 ml/kg, ip). Tap water was again provided during the first 10-min drinking period on the eighth and ninth days and on Day 10 (test day) the animals were given a second opportunity to ingest the sucrose solution.

The purpose of this LiCl conditioning experiment was to assess behaviorally the success of the AP lesions since previous studies have shown that AP lesions block CTA induced by LiCl at this dose range. In this experiment, the ratio of sucrose intake on the test day to intake on the conditioning day was used to measure the degree of conditioning. If an animal drank at least 20% less sucrose on the test day than on the conditioning day, this was taken as evidence that a CTA to sucrose had been acquired. Thus, if an animal had an aversion ratio of 0.80 or less the AP lesion was assumed to be incomplete. On the basis of these ratios several lesioned animals were shifted from their original motion group assignments to different conditions of rotation in an attempt to equate the number of successfully lesioned animals in each of the experimental conditions. (Three sham-operated controls with low aversion ratios were also assigned to motion conditions different from those originally determined by random assignment procedures.)

Conditioning with rotation. Following conditioning with LiCl animals were given tap water during both drinking periods for 4 days. On the motion conditioning day (Day 15) a 4% solution of cider vinegar was substituted for tap water during the first drinking session. Following this drinking session animals were placed into the Plexiglas holding chambers for appropriate treatments. Rotation began 15 min after removal of the cider solution, allowing time for transfer of animals from the home cages to the Plexiglas chambers. Animals assigned to motion treatment conditions were rotated at 150°/s for either 30 or 90 min. Animals in corresponding no-motion control conditions were confined in Plexiglas compartments placed adjacent to the rotation device so that they were subjected to similar noises and vibrations as were rotated animals for either 30 or 90

min. Data on the acquisition and extinction rate of CTA were obtained by providing the cider vinegar solution during the first drinking period on Day 18 (test day) and on Day 21 and Day 24 (extinction trials). Only tap water was offered during either drinking period on all other days. On the conditioning day tap water was presented 15 min after rotation, allowing time for the transfer of animals from the Plexiglas compartments back to their home cages.

After completion of the conditioning tests, animals were deeply anesthetized with sodium pentobarbitol and perfused transcardially with isotonic saline followed by 10% formalin. Brains were stored in 10% formalin for at least 7 days and then transferred to a 30% sugar solution for 2 to 3 days prior to sectioning on a freezing microtome. Coronal sections of 50 μm were cut at the level of the AP, mounted onto gelled slides, and stained with cresyl violet for microscopic examination.

RESULTS

Histology

The extent of each lesion was rated on a 5-point scale with the following descriptive markers: 1 and 2 = incomplete lesions; 3 = subpostrema intact but AP destroyed; 4 = precise lesion of the AP and subpostrema; 5 = AP destroyed but surrounding tissue also damaged (e.g., damage to the nucleus of the solitary tract and/or the fasciculus gracilis). By these criteria lesions were incomplete in 19 animals; data from these animals were not utilized in further analyses. Of the 38 remaining animals, the area subpostrema was left intact in 8 animals, precise lesions of the AP and subpostrema were found in 23 animals, and damage to areas bordering the AP was observed in 7 animals. No evidence of damage to AP was found for rats in the sham control group. Coronal sections of the brainstem showing the AP as seen in a sham-operated animal and in animals with lesion ratings of 2, 4, or 5 are presented in Fig. 1.

Adaptation to Restricted Drinking Schedules

The average consumption of tap water for animals in the three lesion conditions during the 4 days preceding conditioning with LiCl (upper panel) and with motion (lower panel) is presented in Fig. 2. Effects of the experimental variables on the consumption of water during each period of adaptation to the restricted drinking regimen were assessed by computing separate 3 (Lesion Group) × 2 (Drinking Periods) × 4 (Consecutive Days) mixed analysis of variance (ANOVA) with repeated measures on the last two factors.

As expected, animals consumed more water in the first drinking period than in the second during both baseline phases [F's$(1, 93) > 477.94$, p's $< .001$]. The animals exhibited a significant increase in consumption during the adaptation period preceding conditioning with LiCl [$F(3, 279)$

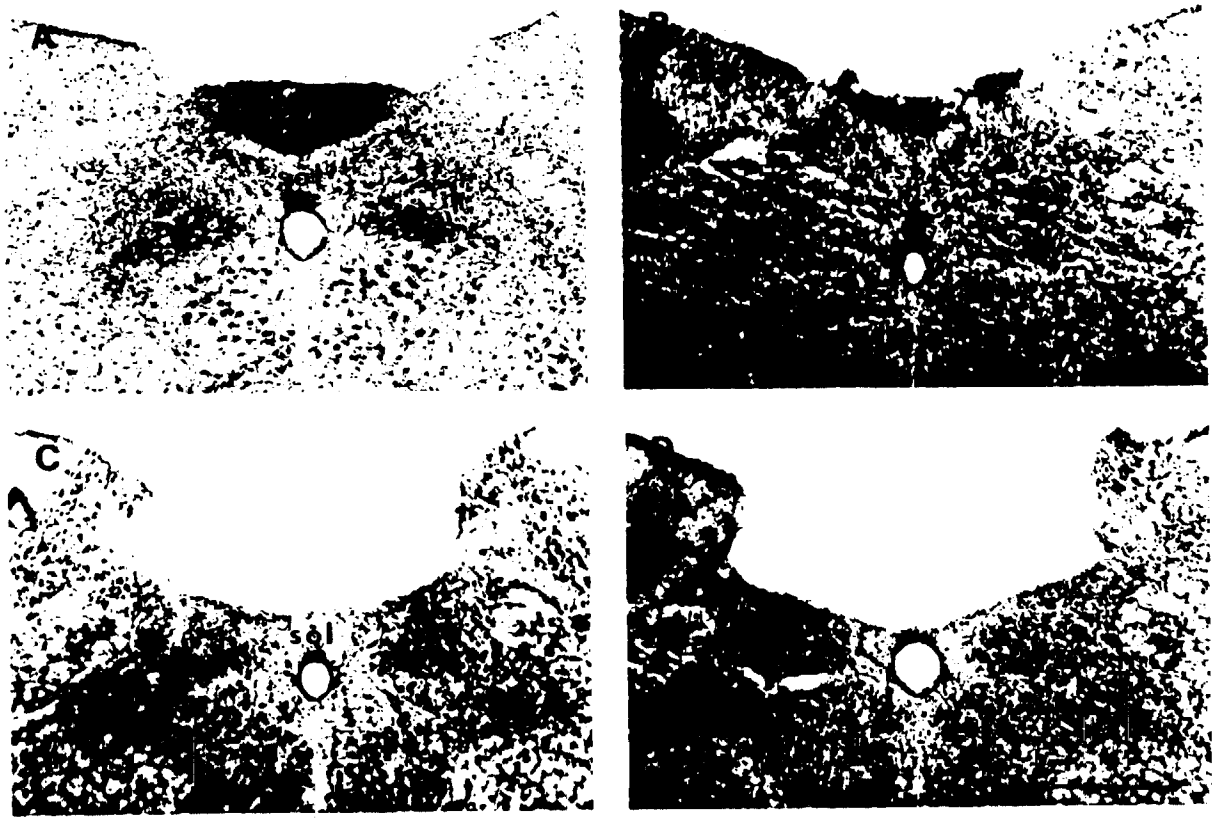

FIG. 1. Coronal sections through the brainstem at the level of the obex showing (A) the AP from a rat in the sham-operated control group; (B) a representative section from an animal with sparing of the rostral AP (lesion rating of 2); (C) an example of a lesion incorporating the AP and subpostrema (lesion rating of 4); and (D) a representative section from an animal with a lesion causing extensive damage to the solitary nucleus in addition to the AP and subpostrema (lesion rating of 5). Calibration bar = 0.5 mm. Abbreviations: ap, area postrema; sol, nucleus of the solitary tract; x, nucleus of the vagus nerve.

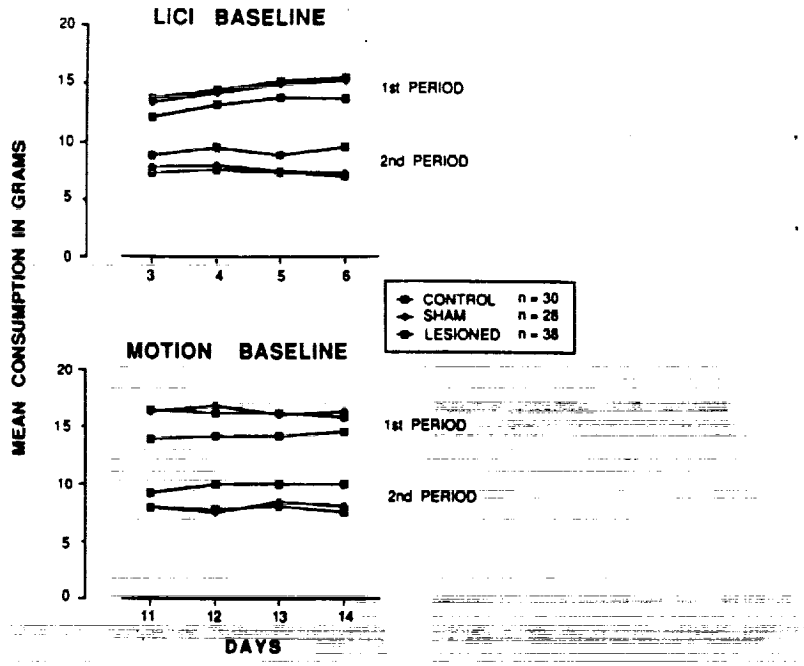

FIG. 2. Mean tap water consumption for animals in the three lesion conditions during the last 4 days of adaptation to limited water access prior to conditioning with LiCl (upper panel) and prior to conditioning with motion (lower panel). The average intake of tap water for each lesion group is shown for the first and second 10-min drinking periods for both the LiCl and motion baseline phases of the experiment.

= 8.93, $p < .001$] as they became adjusted to the restricted drinking regimen (upper panel, Fig. 2). The interaction of Drinking Periods with Days [$F(3, 279) = 8.76$, $p < .001$] in this baseline phase suggests that this increase is due primarily to increased consumption during the first drinking period, as reflected in Fig. 2. In the baseline period preceding conditioning with motion as the US (lower panel, Fig. 2), there was no reliable change in consumption over days ($F < 1$) and there was no interaction of Drinking Periods with Days ($F < 1$), indicating that the animals were fully adapted to the drinking regimen by this point in the experiment.

In both baseline phases there was a reliable interaction of Lesion Groups with Drinking Periods [F's$(2, 93) > 8.76$, p's $< .001$]. These effects reflect the different drinking pattern of the lesioned animals compared to that of the control and sham animals. The total intake of the three lesion groups did not differ (F's < 1), but the average consumption of lesioned animals was consistently less than that of control and sham animals in the first drinking session and consistently more than control and sham

animals consumption in the second drinking session (Fig. 2). This lower fluid consumption by lesioned animals in the first daily drinking session, combined with their compensatory increased intake in the second daily drinking sessions, suggests that ablation of the AP may interfere with physiological mechanisms involved in the initiation of drinking or with regulation of water balance.

Conditioning with LiCl as the US

As a pharmacologic/behavioral method for identifying animals with incomplete lesion of the AP, the strength of CTA produced by LiCl toxicity was examined. The relationship between CTA and the extent of damage to the AP was assessed by computing correlations between the histological ratings for the extent of lesions and the aversion ratios calculated to determine the strength of LiCl-induced CTA. The correlation obtained for these ratios and the 5-point histological ratings for extent of damage to the AP for all 57 of the lesioned animals suggested that there was a weak inverse relationship between CTA to sucrose and the extent of the lesions [$r(50) = 0.32$, $p < .05$]. However, when the analysis was restricted to those 38 animals with lesion ratings of 3, 4, or 5 there was no reliable correlation between the sucrose aversion ratios and the extent of the lesions. This finding indicates that complete ablation of the AP (rating 3) was sufficient to block LiCl-induced CTA (resulting in aversion ratios greater than 0.80) and further damage incorporating the subpostrema (rating 4) or adjacent areas (rating 5) did not reliably alter this effect.

Sucrose intake in the first drinking period: CTA. The average fluid consumption for animals in the three lesion conditions during conditioning with LiCl as the US is presented in Fig. 3. To control for the different drinking pattern induced by AP lesions, fluid consumption was analyzed using repeated measures analysis of covariance (ANCOVA). To determine the reactions of animals to the novel-tasting sucrose solution a 3 (Lesion Group) × 2 (Days 6 and 7) ANCOVA, with repeated measures on the second factor and water consumption on Day 5 as the covariate, was computed. As reflected in the upper panel of Fig. 3, the consumption of sucrose on conditioning day (CD) was not reliably different from consumption of tap water on Day 6 (F's < 1 for Days and the Lesion × Days interaction). The pattern of reduced fluid intake by lesioned animals compared with control and sham animals in the first drinking period was present on the CD, when the animals consumed sucrose solution [$F(1, 92) = 9.434$, $p < .01$], as it was during baseline when the animals drank tap water.

Overall analyses of the conditioning data (upper right panel of Fig. 3) were conducted using a 3 (Lesion Group) × 2 (Days 7 and 10) ANCOVA, with repeated measures on the second factor and water consumption on

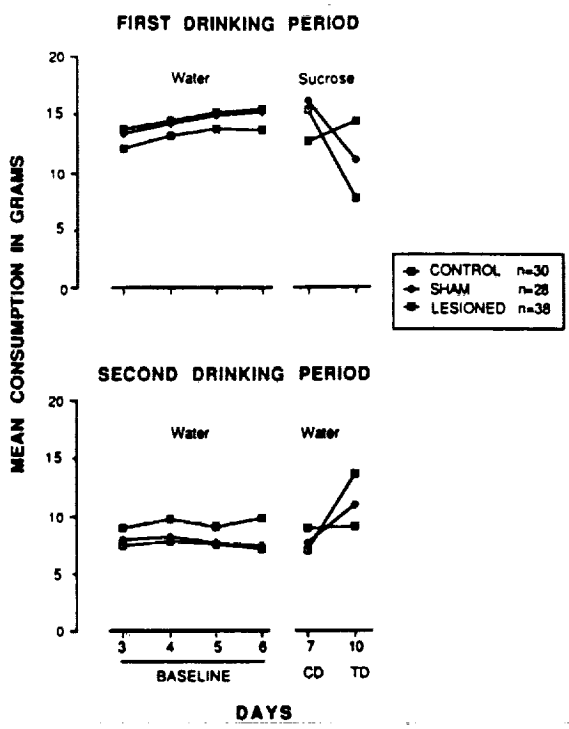

FIG. 3. Mean fluid consumption for animals in the three lesion conditions in both drinking periods of the LiCl conditioning experiment. The data on the left of each panel (reproduced from Fig. 2) represent intake of tap water over the last 4 days of the 6-day adaptation to limited water access during the first (upper panel) and second (lower panel) 10-min drinking periods. The data on the right of the figure represent intake of sucrose (upper panel) or tap water (lower panel) on the LiCl conditioning day (CD) and test day (TD).

Day 6 as the covariate. This analysis revealed a significant effect for the interaction of Lesion Group with Days [$F(2, 92) = 28.770$, $p < .001$], reflecting the increased consumption of sucrose from CD to test day (TD) by the AP-lesioned animals and the decreased intake of sucrose from CD to TD by the control and sham animals. There was also a reliable effect of Lesion Group [$F(2, 92) = 4.219$, $p < .05$], but the main effect of Days was not significant [$F(1, 92) = 3.479$, $p > .05$]. Conditioning effects were examined further by computing the simple effects of Lesion, and the Lesion Group with Days interaction. There was a reliable decrease in the intake of sucrose solution from Day 7 (the CD) to Day 10 (the TD) by the control animals ($p < .001$) and the sham animals ($p < .001$), reflecting CTA produced by pairing the injection of LiCl with the initial consumption of sucrose. The small increase in the average intake of sucrose from Day 7 to Day 10 by the AP-lesioned animals was not

statistically reliable ($p > .05$). Thus, lesioned animals failed to associate the novel-tasting sucrose solution with toxicosis induced by LiCl, thereby confirming the role of the AP in this form of conditioning. As expected, both control animals and sham animals drank less sucrose than did AP-lesioned animals on the TD (p's $< .01$). Although the average intake of sucrose was not different for sham and control animals on the CD ($F < 1$) consumption of sucrose by these two groups was reliably different on TD ($p < .01$), reflecting the stronger CTA to sucrose by animals in the intact control group.

Water intake following injection with LiCl. The overall analysis for the intake of tap water in the second drinking period during conditioning (see Fig. 3, lower right panel) was conducted using a 3 (Lesion Group) $\times$ 2 (Days 7 and 10) ANCOVA, with repeated measures on the second factor and water consumption on Day 6 as the covariate. This analysis indicated reliable effects for Lesion Groups [$F(2, 92) = 8.764$, $p < .001$] and for the interaction of Lesion Groups with Days [$F(2, 92) = 18.380$, $p < .001$]. The interaction of Lesion Groups with Days was examined by computing the simple effects. The consumption of water in this drinking period increased from Day 7 to Day 10 for the sham and control groups (p,s $< .01$), but did not change for the lesioned animals ($F < 1$). Thus, changes in the consumption of water in this second drinking period mirror changes in sucrose intake in the first drinking session; whereas the animals that formed CTA and reduced intake in the first session on Day 10 compensated by increasing intake in the second drinking period, animals which did not form a CTA did not alter intake of water in this second drinking session.

Conditioning with Motion as the US

The average fluid consumption during the two drinking periods in the rotation experiment is shown in Fig. 4. The analyses of data for the two baseline phases and from conditioning with LiCl (presented above) indicated that fluid intake in the two drinking periods is not independent, because animals compensate for variations in consumption in the first period by altering their intake in the second period. Since successful conditioning with rotation would cause decreased consumption in the first drinking period which would lead to increased drinking during the second period, the fluid consumption data for the two drinking periods were analyzed separately. To control for the different consumption pattern exhibited by lesioned animals, data were analyzed using repeated measures ANCOVA followed by analyses of simple effects.

Cider intake in the first drinking period: CTA. The conditioning effects of motion are shown in the data reflecting consumption of cider vinegar in the first drinking period, in the left column of Fig. 4. A marked neophobic response to this solution was seen in all groups on Day 15.

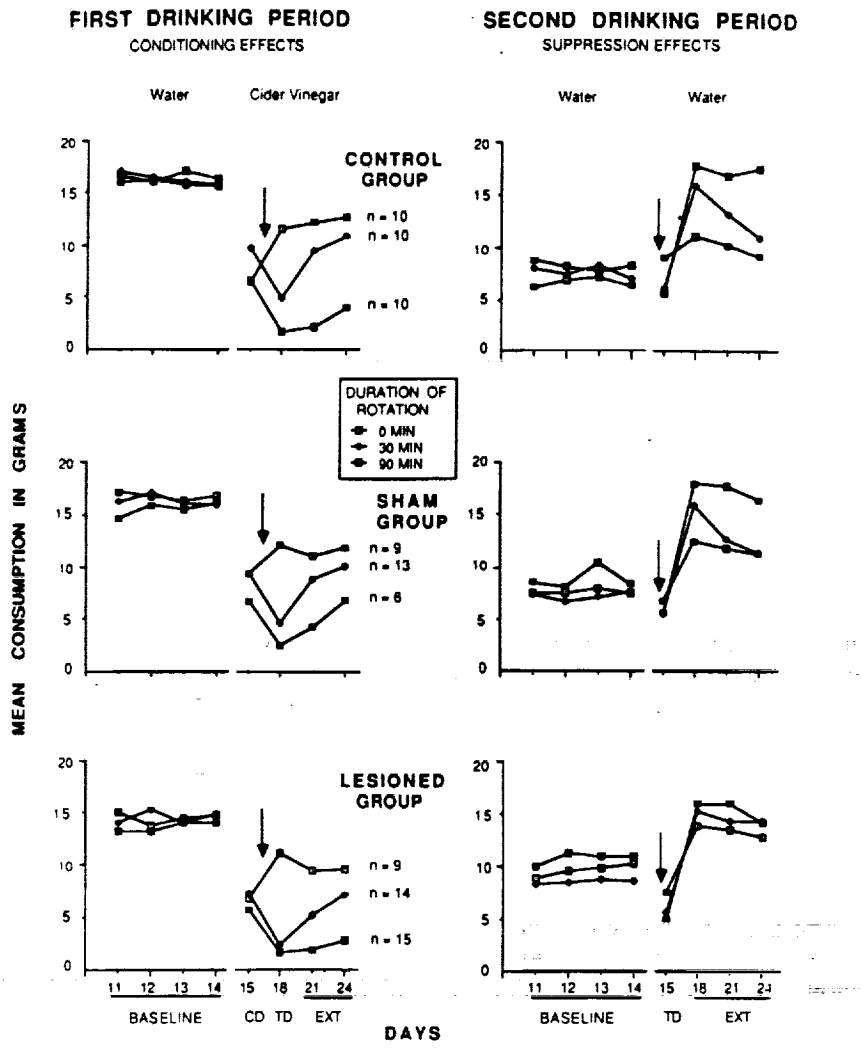

FIG. 4. Mean fluid consumption during both 10-min drinking periods by animals in the nine groups of the rotation conditioning experiment. Data in the left column represent baseline water intake over the 4 days between the LiCl experiment and conditioning with rotation and the cider vinegar intake on conditioning day (CD or Day 15), test day (TD or Day 18), and the extinction (EXT) trials (Days 21 and 24) during the first drinking period. Data in the right column represent water intake during the second drinking periods on these same days. Data for control (upper row), sham (middle row), and lesioned (lower row) animals assigned to each of the three rotation conditions are represented by separate curves in each panel of the figure. The arrows signify that motion occurred after the first (left column) and preceding the second (right column) drinking period on Day 15.

A 3 (Lesion Group) × 2 (Days 14 and 15) ANCOVA, with repeated measures on the second factor and water intake on Day 13 used as the covariate, was computed to evaluate this effect. Significant neophobia was reflected in a reliable effect for Days [$F(1, 92) = 476.165$, $p < .001$]. In addition, there was a reliable difference for Lesion Group [$F(2, 92) = 4.563$, $p < .05$] due to the fact that lesioned animals consumed less cider vinegar than did control or sham animals on Day 15 (p's $< .001$).

The effects of conditioning with motion on cider vinegar consumption on Day 18 (the TD) were first analyzed using a 3 (Lesion Group) × 3 (Motion Duration) ANCOVA, with consumption on Day 15 (the CD) as the covariate. The overall analysis reflected a significant main effect for Motion Duration [$F(2, 86) = 112.849$, $p < .001$], but no reliable effect for Lesion Group and no reliable interaction of Lesion Group × Motion Duration (F's < 1). Thus, the acquisition of CTA reflected in reduced intake by the groups exposed to rotation was not different for the three lesion groups. Subsequent analyses of the simple effects of Motion Duration indicated that both the 30- and 90-min rotation groups developed significant CTA to the cider vinegar as compared with the no-motion animals (p's $< .001$; see the left panels of Fig. 4). However, the magnitude of CTA to the cider vinegar solution, reflected by consumption on Day 18, did not differ for the 30-min and 90-min motion duration groups ($p > .10$). These results indicate that off-vertical rotation at 150°/s for either 30 or 90 min is an adequate US for producing CTA and, it is also clear that lesioning of the AP does not block acquisition of CTA induced by these conditions of motion. These results also suggest that the intitial acquisition of motion-induced CTA is not affected by the intensity of the motion US.

Cider intake in the first drinking period: Extinction. To evaluate the effects of lesion condition and motion duration on the rate of extinction of CTAs a 3 (Lesion Group) × 2 (30- or 90-min Motion Duration) × 2 (Days) mixed ANCOVA, with repeated measures on the last factor and Day 15 used as the covariate, was computed for cider vinegar intake on Days 21 and 24. The overall analysis indicated that the effect of Motion Duration was reliable [$F(1, 61) = 43.505$, $p < .001$], reflecting the slower rate of extinction of animals rotated for 90 min as compared to those in the 30-min rotation groups. There was also a significant effect for Lesion Groups [$F(2, 61) = 7.876$, $p < .001$], but no reliable effect for Days ($F < 1$) and no significant interactions. Analysis of the simple effects for Lesion Groups indicated that the rates of CTA extinction did not differ significantly for animals in the intact control and sham-lesioned groups ($p > .17$). However, the AP-lesioned animals had reliably slower rates of extinction of CTAs than did the sham or intact control groups (p,s $< .05$). Thus, in contrast to results obtained from the analyses of CTA acquisition as reflected by consumption of cider on Day 18 only, the

results from this analysis suggest that the magnitude of the motion US does significantly affect the rates of CTA extinction. Furthermore, lesioning of the AP also slows the rate at which animals extinguish motion-induced CTAs to a cider vinegar solution (see the left panels of Fig. 4). This latter finding is compatible with the notion that AP lesions enhance the magnitude of CTAs induced by motion in rats (Ossenkopp, 1983).

Suppression of Postrotational Drinking

The suppressive effects of motion on water consumption on Day 15 are shown in the right column of Fig. 4. To evaluate postrotational suppression of drinking on Day 15, a 3 (Lesion Group) × 3 (Motion Duration) ANCOVA was computed, using water intake in the second drinking period on Day 14 as the covariate. This analysis revealed a significant effect for Motion Duration [$F(2, 86) = 7.438$, $p < .001$]. The effect of Lesion Group [$F(2, 86) = 2.196$, $p < .20$] and the Lesion × Motion Duration interaction ($F < 1$) were not significant. Simple effects computed for Motion Duration indicated that, compared with no-motion animals, both 30 min ($p < .01$) and 90 min ($p < .001$) of rotation at 150°/sec were sufficient US for producing a significant postrotational suppression of drinking. The magnitude of this postrotational suppression of drinking was not reliably different for animals rotated for 30 min versus those rotated for 90 min ($F < 1$). These overall findings support the proposal that drinking suppression can be produced in the rat by rotary stimulation, although the duration of the motion US did not affect this measure. It is also apparent from these results that AP lesions in rats do not prevent the suppression of postrotational drinking.

Fecal Boli during Rotation

The data for boli counts were first evaluated by computing a 3 (Lesion Group) × 3 (Motion Duration) ANOVA. This analysis revealed no reliable effects of either Lesion Condition ($F < 1$) or Motion Duration [$F(2, 87) = 2.680$, $p < .10$]. There was also no significant Lesion × Motion Duration interaction [$F(4, 87) = 1.148$, $p > .25$]. With the exception of the intact control group which was simply confined for 90 min, the mean number of fecal boli was always higher for animals in the confinement plus rotation conditions than for animals which were confined in the rotation apparatus but not rotated. Although these results suggest that defecation in the rat may be increased by rotational stimulation, the numerical increases in fecal boli in response to motion were very small. To further evaluate these data, animals in the no-motion conditions were subdivided into two groups, depending on whether they were confined in the Plexiglas containers for 30 or 90 min. This resulted in the formation of 12 groups formed by the factorial combination of three lesion conditions (control, sham, or lesioned), two motion conditions (no-motion or motion),

and two confinement durations (30 or 90 min). A 3 (Lesion Condition) × 2 (Motion Condition) × 2 (Confinement Duration) ANOVA was then computed on the boli data. The only reliable effect found with this analysis was for the Confinement Condition [$F(1, 84) = 13.238$, $p < .001$], indicating that boli counts increased as time of confinement in the Plexiglas holding cages increased. These results indicate that the AP in the rat does not mediate the response of defecation during motion and weaken the validity of the claim that increased production of fecal boli during rotation is a reliable index of motion sickness in the rat.

DISCUSSION

The results of the LiCl experiment in this study confirm prior reports that thermal cauterization of the AP disrupts CTAs induced by LiCl (Hartley, 1977; McGlone et al., 1980; Rabin, Hunt, & Lee, 1983; Rauschenberger, 1979; Ritter et al., 1980). The consistency of this finding indicates that this procedure can serve as a useful pharmacologic validation of successful lesion of the AP, such as was done for screening AP-lesioned animals in this study for later use in the rotation experiment. Furthermore, results of the correlational data between strength of aversions and extent of damage to the AP suggest that it is the AP and not immediately adjacent structures which mediates development of LiCl-induced CTA.

We also found that lesioning of the AP in rats resulted in a long-term reduction in body weight (presurgical weights were never attained in the 34 days postsurgery before conditioning procedures began), which is in agreement with results reported by other investigators (Berger et al., 1973; Carlisle & Reynolds, 1961; Coil & Norgren, 1981). Similar effects of AP lesions are reported for species in which emesis occurs, although recovery of appetite and interest in food occurs within 2–3 days in monkey (Brizzee et al., 1980) and within a week or so in cats (Borison & Borison, 1986). Although food intake was not monitored in this experiment, the chronic weight reduction in AP-lesioned rats seems more likely to be due to alterations in food consumption than to altered fluid intake. Although lesioned rats consistently drank less water in the first daily drinking periods and more water in the second drinking period than did animals with an intact AP, the AP-lesioned, sham, and control rats did drink comparable overall amounts of water on baseline days. The reasons for this alteration in the pattern of drinking are not clear, but previous investigators have also suggested that the AP plays a role in the regulation of drinking behavior (Edwards & Ritter, 1982).

In contrast to the findings on LiCl-induced CTA, lesions of the AP had no effect on conditioning, as measured by the strength of initial CTA acquisition, when motion served as the US. The AP-lesioned rats in this experiment developed CTA to cider which in magnitude was comparable to that acquired by sham-lesioned and intact control animals. However,

as assessed by the rate of extinction of CTAs induced by rotation, lesions of the AP do affect the duration of motion-induced CTA. The CTAs to cider extinguished more slowly for lesioned animals than they did for sham or intact control animals. This finding is generally in agreement with that of Ossenkopp (1983), who reported that AP lesions enhanced formation of motion-induced CTAs. The finding that animals rotated for 90 min developed more enduring CTAs, as measured by rates of extinction, than did those rotated for 30 min also supports the notion that CTAs induced by this US are dose-dependent (Green & Rachlin, 1976), as they are when drugs are used as the US (Nachman & Ashe, 1973; Rabin et al., 1987; Rauschenberger, 1979). Overall, it seems quite clear from the results of this study and the studies by Hartley (1977) and Ossenkopp (1983) that the AP is not a critical neural structure mediating motion-induced CTA in the rat. Furthermore, the failure of AP lesions to block CTA induced by any of the four motion parameters used in these studies indicates that the AP does not mediate motion-induced CTA in a dose–response fashion as this structure appears to do for drug-induced CTA (Rabin et al., 1987; Rauschenberger, 1979).

Analysis of data of drinking suppression confirms the previous report that rotary stimulation causes suppression of postrotational drinking (Haroutunian et al., 1976). However, as revealed by the lack of any differential suppression between animals rotated for 30 versus 90 min, this measure may not be sensitive to the duration or intensity of vestibular stimulation. Results from the current experiment also indicate that AP lesions do not attenuate suppression of postrotational drinking.

Results obtained on defecation accompanying confinement and/or motion in this experiment raise questions about the reliability and validity of this measure as an index of motion sickness in the rat (Ossenkopp & Frisken, 1982). Since animals confined in the holding apparatus for 90 min exhibited more defecation than did animals confined for 30 min, regardless of whether or not they were rotated, it seems reasonable to suggest that increased levels of defecation may simply reflect emotionality (Hall, 1934) from the stress of confinement. Clearly, the AP does not play a role in the elaboration of this response to motion since lesioned, sham, and intact control animals did not differ in their levels of defecation across either of the two rotation conditions used in this study. This finding is consistent with those found in AP-ablated cats, where subemetic signs of motion sickness including salivation, panting, urination, and defecation were all present after lesioning of the AP (Borison & Borison, 1986).

The overall results of the present studies indicate that AP lesions in the rat do not prevent (1) formation of CTA to a cider solution paired with motion, (2) the suppression of drinking following exposure to motion, or (3) amount of defecation during exposure to motion; three measures

proposed as species-relevant measures of motion sickness in the rat. Although additional postrotational behavioral measures, including pica (Mitchell et al., 1977) and reduction of bar pressing for food reward (Riccio & Thach, 1963), have been proposed as pertinent indices of "illness" or "motion sickness" in the rat, we know of no studies showing that these measures depend upon physiological mechanisms or have neural pathways in common with those involved in frank motion sickness. Reduction of spontaneous locomotor activity after rotation has also been proposed as a behavioral index of motion sickness in the rat (Eskin & Riccio, 1966). Vestibular damage reduces the effects of motion on spontaneous activity (Riccio, Igarashi, & Eskin, 1967), consistent with findings from similar studies in species capable of emesis (Meek, Graybiel, Beischer, & Riopelle, 1962). However, spontaneous activity as a measure of motion sickness in rats is still questionable since there is no way to ensure that decreases in activity are due to sickness per se, or due to a lack of muscle coordination or dizziness which may be produced by rotation independent of other physiological effects comprising the prodromal symptoms of motion sickness.

At the time these studies were initiated conventional wisdom held that the AP was the locus of the chemoreceptor trigger zone which mediated the emetic response to both motion and drugs (Borison, 1974; Borison, Borison, & McCarthy, 1984; Borison & Wang, 1951, 1953; Wang & Borison, 1950; Wang & Chinn, 1954), as well as to X-irradiation (Brizzee, Neal, & Williams, 1955, Wang, Renzi, & Chin, 1958). Similarly, CTA induced by blood-borne toxins (Berger et al., 1973; Coil & Norgren, 1981; McGlone et al., 1980; Rauschenberger, 1979; Ritter et al., 1980) or by X-irradiation (Ossenkopp & Giugno, 1985; Rabin et al., 1983) are attenuated or abolished by lesion of the AP and data suggest that a humoral factor resulting from exposure to radiation may mediate formation of CTA (Hunt, Carroll, & Kimeldorf, 1965, 1968). Although the possible chemical substances eliciting vomiting have remained elusive, it has been proposed that a humoral factor released during motion triggers the emetic reflex (Crampton & Daunton, 1983; Wang & Chinn, 1956). Hence, the idea that humoral factors released during rotational stimulation in the rat might underly formation of motion-induced CTA and be mediated by the AP was considered.

The data reported here as well as those of Ossenkopp (1983) suggest that if a humoral factor is produced by motion in the rat resulting in formation of CTA, the AP is not the site of chemoreceptors mediating this response. Recent experiments in the cat (Borison & Borison, 1986; Corcoran, Fox, Brizzee, Crampton, & Daunton, 1985) have also reported contradictory findings from earlier work in dog (Wang & Chinn, 1954) and monkey (Brizzee et al., 1980) regarding the function of the AP in mediation of motion sickness. Both of these studies found that while AP

lesions made cats refractory to drug-induced emesis, ablation of the AP did not prevent motion-induced vomiting. Thus, it appears that for both cat and rat, if a pathophysiologic humoral factor is being released by motion, the AP is not the neural structure mediating the resultant adverse consequences.

Further research is needed to determine which, if any, of the neural mechanisms known to mediate drug-induced CTA are involved in motion-induced CTA. One likely candidate is the vagus, since any internal aversive state produced in the rat by rotational stimulation could be acting through peripheral gastrointestinal mechanisms, much like intragastric copper sulfate. This peripherally acting emetic produces strong CTA in the rat (Nachman & Hartley, 1975), produces emesis in animals with lesions of the AP (Borison & Wang, 1953), but is ineffective for producing CTA in vagotomized rats when given orally or intragastrically (Coil et al., 1978; Rauschenberger, 1979). However, although a study on motion-induced CTA in vagotomized rats would be informative, even if such intervention prevents acquisition of motion-induced CTA any analogy with "motion sickness" will be equivocable, since nausea and vomiting produced by motion are still present after either gut denervation or total abdominal evisceration (Borison & Wang, 1953; Wang, Chinn, & Renzi, 1957).

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