FINAL REPORT

for the Project

Self-Motion Perception and Motion Sickness.

NASA -Ames Cooperative Agreement (NCC 2-167).

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I. INTRODUCTION

Motion sickness typically is considered a bothersome artifact of exposure to passive motion in vehicles of conveyance. This condition seldom has significant impact on the health of individuals because it is of brief duration, it usually can be prevented by simply avoiding the eliciting condition and, when the conditions that produce it are unavoidable, sickness dissipates with continued exposure.

However, unavoidable motion sickness is a malady that can have significant effects on the performance of affected individuals. Because the susceptibility of individuals to motion sickness cannot be predicted with precision, some individuals can be seriously affected if they are required to work in an environment that produces motion sickness. The affliction of individuals of unknown susceptibility by this malady can become important when sickness could arise during periods where complicated but necessary performance is demanded. The occurrence of space motion sickness during entry into or return from space flight is one possible case of this type. Important human activities are required during launch and landing of the Space Shuttle, precisely the times when "space sickness" can occur.

There is some debate about the equivalence of motion sickness produced in ground-based studies and "space sickness". However, ground-based studies provide certain benefits over flight studies. Ground-based studies can be conducted at considerable cost savings, the necessary control conditions can be included with experimental rigor, and the appropriate number of subjects an be used to address the experimental questions. Because there are numerous similarities between motion sickness and space sickness, it appears that better knowledge of motion sickness could significantly benefit the understanding and future study of space sickness.

The studies conducted in this research project examined several aspects of motion sickness in animal models. A principle objective of these studies was to investigate the neuroanatomy that is important in motion sickness with the objectives of examining both the utility of putative models and defining neural mechanisms that are important in motion sickness.

II. PRINCIPLE AREAS of INVESTIGATION

For purposes of exposition, the studies and research findings have been classified into four categories. These categories are used to organize the presentation of the results and to facilitate the discussion of motion sickness in animal models. Thus, the results of these studies of motion sickness in animal models are organized with the following categories: (a) behavioral measures of the phenomenon, (b) stimuli that are effective for producing the phenomenon, (c) neuroanatomical structures and physiological events that are related to the phenomenon, and (d) differences between species in the elicitation of the phenomenon.

The first two of these categories, behavioral measures and effective stimuli, were addressed in the initial experiments because results from these two categories of studies were fundamental to planning other experiments. After behavioral measures had been subjected to initial validation and appropriate parameters were established for eliciting stimulation, studies were conducted to examine neural structures and physiological events that were related to motion sickness. As studies related to these questions progressed, the issue of species differences in the response began to arise and this was subjected to both retrospective analysis and direct experimental examination.

A. Behavioral Measures

Frank sickness, or vomiting, is the only universally accepted response that defines motion sickness in all species. None-the-less, many other responses (e.g., pallor, increased salivation, defecation) are commonly considered to be part of the general syndrome of motion sickness. In studies of motion sickness in humans nausea, a measure that is obtained only by self report, commonly is used as a prominent prodromal symptom of sickness.

Nausea and other prodromal symptoms that are detected by self report can be used in humans, but there are no reliable methods for obtaining self reports of symptoms in animals. Because of this, two alternative methods are used to assess the development of motion sickness in animal models. One system is to use rating scales based on the assessment of various responses that are putative prodromal symptoms. Such scales have been developed for use with cats, squirrel monkeys and chimpanzees (Fox, 1992). An extensive discussion of rating scales with animals is presented by Daunton (1989). The second strategy is to use specific responses (e.g., conditioned taste aversion) that are thought to be related to neural or physiological mechanisms that underlay motion sickness. This strategy has been used with animals that do not have a

complete emetic reflex and as multiple or supplemental indices of sickness (Ossenkopp & Ossenkopp, 1985).

Experimental studies were conducted to evaluate pica, conditioned taste aversion (CTA), and anorexia as putative measures of motion sickness. Pica was proposed as a measure of gastric distress and motion sickness by Mitchell (1977). This response was selected for assessment because it results as increased responding rather than as reduced responding which is common with many of the other putative measures for animal models (Fox, 1990). CTA is the measure most commonly asserted and best documented for assessing nausea and sickness in animals. Anorexia commonly occurs with motion sickness in humans and anecdotally also in animals that have a complete emetic reflex. Consequently this measure was evaluated in several experiments.

1. Pica.

Pica was studied in rats with sickness induced by vertical and off-vertical rotation at 150^0/s. No reliable pica response was produced by this treatment when appropriate groups were used to control for confounded effects of food deprivation. Pica could be induced by exposing food-deprived animals to motion, but food deprivation itself also induced pica.

This result is in contrast to data reported by Mitchell (1977). It should be noted, however, that studies showing pica in rats have used intense motion stimuli (e.g., 7500^0/s) or severe gastric irritants as inducing treatments. The purpose of these preliminary studies in this project was to investigate whether pica could be produced in rats using moderate motion conditions that were representative of treatments known to produce motion sickness in man and other animals. In that regard the answer to the experimental question was that pica was not a useful measure.

2. CTA.

It has long been recognized that CTA can be produced by numerous interventions, including motion, that are known to produce gastric distress in humans and animals. This relationship between CTA and "internal malaise" has led to widespread interest in using CTA to assess several forms of sickness arising from gastric disruption in animal models. Several observations have indicated that CTA may be a useful measure of motion sickness in animal models. Important among these is the demonstration that an intact vestibular system is equally crucial for the production of motion sickness in man and CTA in animals. This, and other relevant relations are reviewed in Fox (1990).

In this project it was demonstrated that CTA is produced by exposure to several forms motion of moderate magnitude that are known to produce vomiting in animals with a complete emetic reflex and in humans (Fox & Daunton, 1982; Fox *et al.*, 1984). However, the precise utility of CTA as a measure of motion sickness remains to be described. A paramount concern in this regard is that the relationship between vomiting and CTA is not isomorphic in either cats or squirrel monkeys (Fox *et al.*, 1990). Because CTA is not precisely related to the universal symptom of motion sickness in species with a complete emetic reflex, the validity of CTA as a prodromal symptom in these species and as a primary index of sickness in rodents that fail to vomit is uncertain.

3. Anorexia.

Two experiments were conducted to directly test anorexic and conditioning effects of motion. These effects were assessed by exposing animals to either off-vertical rotation or to parabolic flight using 15 parabolas in a Lear jet. Anorexia was assessed in rats permitted to feed for 2h per day with exposure to motion on test days occurring just prior to the feeding session. CTA was conducted using procedures described elsewhere (Fox & Daunton, 1982).

The mean daily consumption of food in the experiment using off-vertical rotation is shown in Figure 1. Food consumption increased and body weight decreased over the initial days of adaptation to the restricted feeding regimen (2 h/day) until intake stabilized by about Day 11. Using Day 19 (the day preceding exposure to motion) as a baseline, anorexia was present on the day of exposure to motion (on Day 20, $ps<.001$) but intake on Days 21 & 22 did not differ from baseline ($p>.39$). Body weight was suppressed on each of the three days following exposure to motion ($ps<.003$).

The mean daily consumption of food in the experiment using parabolic flight is shown in Figure 2. Again, food consumption increased as the animals adapted to the restricted feeding schedule (2 h/day) with body weight reflecting an initial decrease (first 5 days) followed by a normal tendency to increase. On Day 12 the rats were transported to the flight line and loaded on the airplane to determine whether this activity would affect the dependent measures. No effects where seen on either food intake or body weight with this procedure ($F<1$). With Day 18 as a

baseline, anorexia was present immediately after the flight (Day 19) and 48 h later (Day 20). Food consumption 72 h after the flight did not differ from that preceding the flight ($p>.80$). Food intake was suppressed immediately and 24 h following flight ($ps<.002$) but by 48 h after the flight food intake did not differ from the baseline ($p>.11$).

The effects if parabolic flight on CTA are shown in Figure 3. Intake of flavored fluid by animals in the Control and Flight groups did not differ prior to the flight, but in the test following parabolic animals in the Control group consumed more fluid than did the animals in the Flight group ($p<.001$). It should be noted that the strength of conditioning is rather weak. There was no significant suppression of intake in animals exposed to flight. Rather, these animals failed to increase intake as was seen in animals from the Control group. Thus, a release from neophobia in Control animals with a failure to observe this release in Fight animals appears to create this difference.

The observed suppression of food intake could be a form of anorexia similar to that produced during prolonged exposure of animals to hyper-gravity during centrifugation, or to hypo-gravity during orbital flight. The coincident emergence of anorexia and CTA is consistent with the proposal that the rats became motion sick during the altered gravity during the brief exposures to parabolic flight. The magnitude of flight-induced anorexia is as great as, or greater than that produced by a form of passive, cross-coupled stimulation that is very provocative for humans. A)Anorexia following parabolic flight was present for 48 h while that produced by rotation was absent after only 24 h.

Informal observations regarding anorexia were conducted in both cats and squirrel monkeys to begin examination of this effect in animals with a complete emetic reflex. Both cats and squirrel monkeys were repeatedly observed to eat the food normally contained in their diets (cat food and bananas respectively) within a few minutes (e.g., less than 5 min) after vomiting. These observations indicate that anorexia is not necessarily present when the universally accepted indicator of motion sickness occurs. These effects have not been satisfactorily resolved as not formal experiments were conducted to test this issue further.

B. Effective Stimulus Parameters

The specific parameters of stimulation that effectively produce motion sickness in animal models was examined in several experiments. Two general results were documented with these experiments: (a) motion sickness can be produced in rodents, cats, and squirrel monkeys with moderate stimulation that is comparable to that which elicits sickness in humans; (b) stimuli that are very provocative for one species may be quite ineffective for producing sickness in another species.

Conditioned aversion can be produced in rodents with rotational stimulation that is of the magnitude that produces vomiting in squirrel monkeys, chimpanzees, and humans (Fox & Daunton (1982); Fox *et al.*, 1984). Further, this same stimulation can produce CTA in both cats and squirrel monkeys (Fox *et al.*, 1990). Thus, it is clear that conditioned aversions do not depend on severe motion challenges.

Detailed examination of eliciting stimuli in squirrel monkeys reflected that exposure to stimuli of increasing intensity to humans also elicited more severe sickness in the monkey (Fox *et al.*, 1982). An important result in this study, however, was the finding that stimuli that are extremely provocative for humans are effective for the squirrel monkey only when there is a requirement for the animal to maintain posture. When animals were exposed to provocative stimuli (cross-coupled stimulation) while movement was restricted at both the neck and waist, the same stimulus that elicited sickness with waist restraint only no longer was effective. Thus, it appears that a requirement for postural control during passive motion is necessary if motion sickness is to be elicited.

Examination of the role of vision in motion sickness produced the first demonstration of vomiting in cats and squirrel monkeys by visual stimulation alone (Daunton *et al.*, 1985). Sickness induced by visual stimulation alone is known in man and is very disruptive in certain instances (e.g., "simulator sickness), but this has not been shown previously in an animal model. With regard to the problem of prediction, it was shown in this research that animals more prone to sickness by passive, whole-body stimulation also were more likely to become sick by optokinetic stimulation alone.

C. Neuroanatomy and Physiology

1. Vasopressin.

Vasopressin (AVP) is elevated in humans during reports of nausea and following vomiting (see Fox, 1992 for a review). Plasma AVP is dramatically elevated in cats following vomiting but the resting level of AVP in blood plasma

does not differ among cats that are selected to be highly susceptible or very resistant to linear acceleration. On the other hand, AVP in cerebrospinal fluid (CSF) is not elevated following motion sickness, but resting levels of AVP in CSF are lower in animals that vomited during motion than in those animals which did not vomit (Fox *et al.*, 1987). The precise mechanism for the release of AVP during motions sickness could not be determined. Systemic injection of AVP at dosages calculated to produce levels equivalent to those observed following vomiting failed to produce vomiting or to influence the onset of vomiting in cats that were susceptible or resistant to to linear acceleration (Unpublished Data).

2. Area Postrema.

Experiments using the lesion technique to examine the role of the area postrema showed that: (a) The area postrema is not involved in CTA that is produced by motion in rats (Sutton *et al.*, 1988); (b) Neither CTA nor vomiting are crucially dependent on the area postrema in either cats or squirrel monkeys (Fox, Corcoran & Brizzee, 1990). In combination with work by Borison and Borison (1986), these findings contributed to a reevaluation of the role of the area postrema in vomiting induced by motion (Daunton *et al.*, 1987). several authors have now proposed theories which include several additional brainstem and/or circumventricual structures in the emetic response (see Fox, 1992 for references).

3. Vagus Nerve.

A possible role for the vagus nerve in responses to motion is implied by results showing that the vagus nerve is crucial to CTA induced in rodents by exposure to motion (Fox & McKenna, 1988). Combined with other research, this finding shows that both vestibular and gastric neural systems contribute to the formation of CTA when motion is the stimulus. The specific mechanism by which gastric circuitry functions is unknown (Fox, Sutton, & McKenna, 1988), but we did provide evidence indicating that gastric afferents of the rat remain active for an extended period following brief physiological stimulation (Nijiima *et al.*, 1987; 1988).

4. Immunocytochemistry.

Preliminary evidence indicating a role for the vagus nerve either in motion sickness or in adaptation to stimuli producing motion sickness has been shown. Using immunocytochemistry we showed that the distribution pattern of GABAergic terminals in the area postrema, nucleus tractus solitarius, area sub-postrema, and gelatinous nucleus closely resembles that of vagal afferent projections (D'Amelio *et al.*, 1988). In addition, the depletion of GAD immunoreactive in these areas after electrical stimulation of the vagus nerve seems to confirm that at least part of the GABAergic activity shown here corresponds with vagal afferents. The additional demonstration of substance P immunoreactivity in this study implies there may be important neuromodulatory functions mediated by neuropeptides.

D. Species Differences

While conducting the studies discussed above to evaluate behavioral measures of and effective stimuli for motion sickness it became increasingly obvious that stimuli that elicited sickness and the syndromes observed in different species varied greatly. For example, linear acceleration, particularly earth-vertical acceleration, is an especially effective stimulus for eliciting motion sickness in cats while vertical axis rotation is remarkably noneffective. On the other hand, vertical axis rotation is very provocative for the squirrel monkey while linear acceleration has only minimal effectiveness with this species.

Species differences can occur in rather closely related species where similar reactions to stimuli might be expected. For example, although vertical axis rotation is very provocative for squirrel monkeys, we were unable to make rhesus monkeys motion sick with this stimulus (Corcoran, Fox, & Daunton, 1990). In fact, both anecdotal and experimental evidence indicate that the rhesus monkey is highly resistant to motion sickness. Workers in the Russian space program have reported "space sickness" but there have been no well controlled studies reporting on these effects (see Daunton, 1990 for a review of these points).

Difference of this type complicate the selection of appropriate animal models for studying the emetic reflex in general and motion sickness or the space adaptation syndrome in particular. Animal models will be crucial to the discovery and understanding of neurophysiological mechanisms of these phenomena, but considerable research will be required before answers come forth.

III. CONCLUSIONS

All of the behavioral responses that have been examined as measures of motion sickness in animals are less than ideal. The only response that is universally accepted as a valid measure is vomiting. On initial consideration this appears to be a serious weakness in this area of research. However, it should be recognized that no other measures have been universally accepted for the assessment of motion sickness in humans. The most commonly used additional measure in human studies is nausea, but the assessment of nausea, even in humans can be quite inaccurate. Furthermore, there are no recognized physiological correlates of nausea in humans, further complicating the assessment of this response in animals.

The issue of prediction of susceptibility to motion sickness also is difficult. Significant attention has been directed to the problem of prediction in humans with only minimal success. While we found some evidence for predictive value in the level of AVP in CSF, this effect was not highly predictive and significant work would be required to understand this relationship adequately. As is the case in humans, plasma AVP was dramatically elevated in cats following vomiting, but there was no evidence in this research to indicate that the level of system AVP was predictive of susceptibility to motion sickness.

It is now abundantly clear that previous conceptions of the area postrema as a vomiting center in motion sickness were premature and incorrect. This conceptualization arose, in part, from over-interpretation of lesion experiments before many of the techniques of neuroscience that are in common use today were available. With present knowledge, many workers now propose by that the emetic reflex is mediated via circuitry in several circumventricular and brainstem regions. Important work remains to provide understanding of the specific neural mechanisms of the response that is so important in disease and travel by modern conveyances.

A general hypothesis that was developed during the course of this research project is that motion sickness is a phenomenon that may reflect only one of the outcomes of the more general effects of adaptation to unusual environmental conditions. Motion sickness arises when organisms are subjected to passive motion that results in atypical linear forces on the vestibular system. Passive motion of this type can elicit significant and pervasive adaptive responses in many systems other than the emetic reflex (e.g., motor coordination, postural reflexes, etc.). Consequently, it is reasonable to expect that many systems may be undergoing significant changes (i.e., adaptations) during periods when motion sickness occurs. In fact, one way to avoid motion sickness is to select a behavior that prevents adaptation of motor systems such as lying down or going to sleep. In this regard it should be noted that motion sickness occurred in squirrel monkeys only when there was a necessity to maintain posture. This hypothesis would suggest that motion sickness might simply be an unfortunate outcome of normal processes of adjusting the neuromuscular system to new, atypical environmental conditions. Although the specific mechanisms that may be involved in such processes are obscure at this time, discovery of the physiology and neural changes that underlie adaptation may predict the mechanisms that elicit motion sickness.

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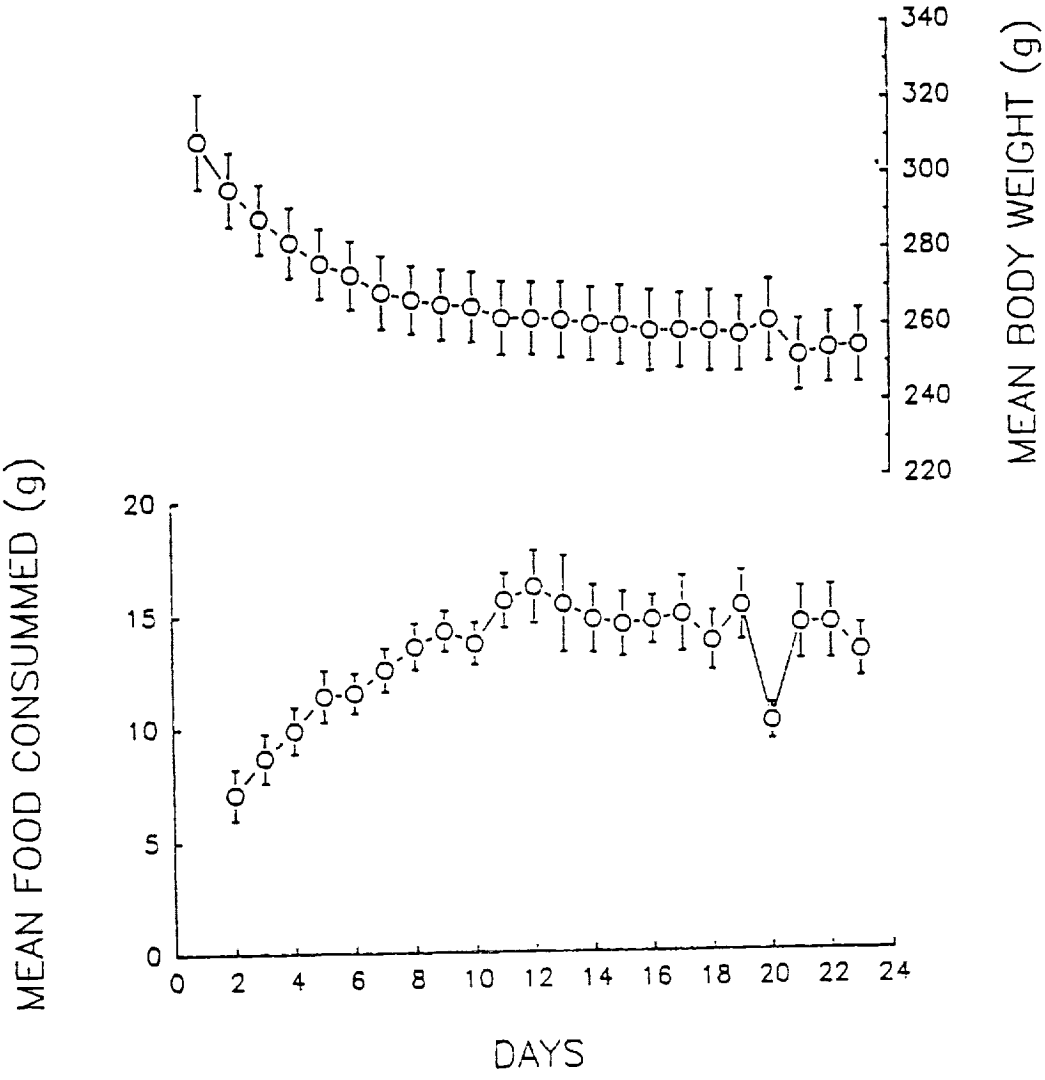

Figure 1. Mean Food Intake and Body Weight by Rats Exposed to Off-Vertical Rotation.

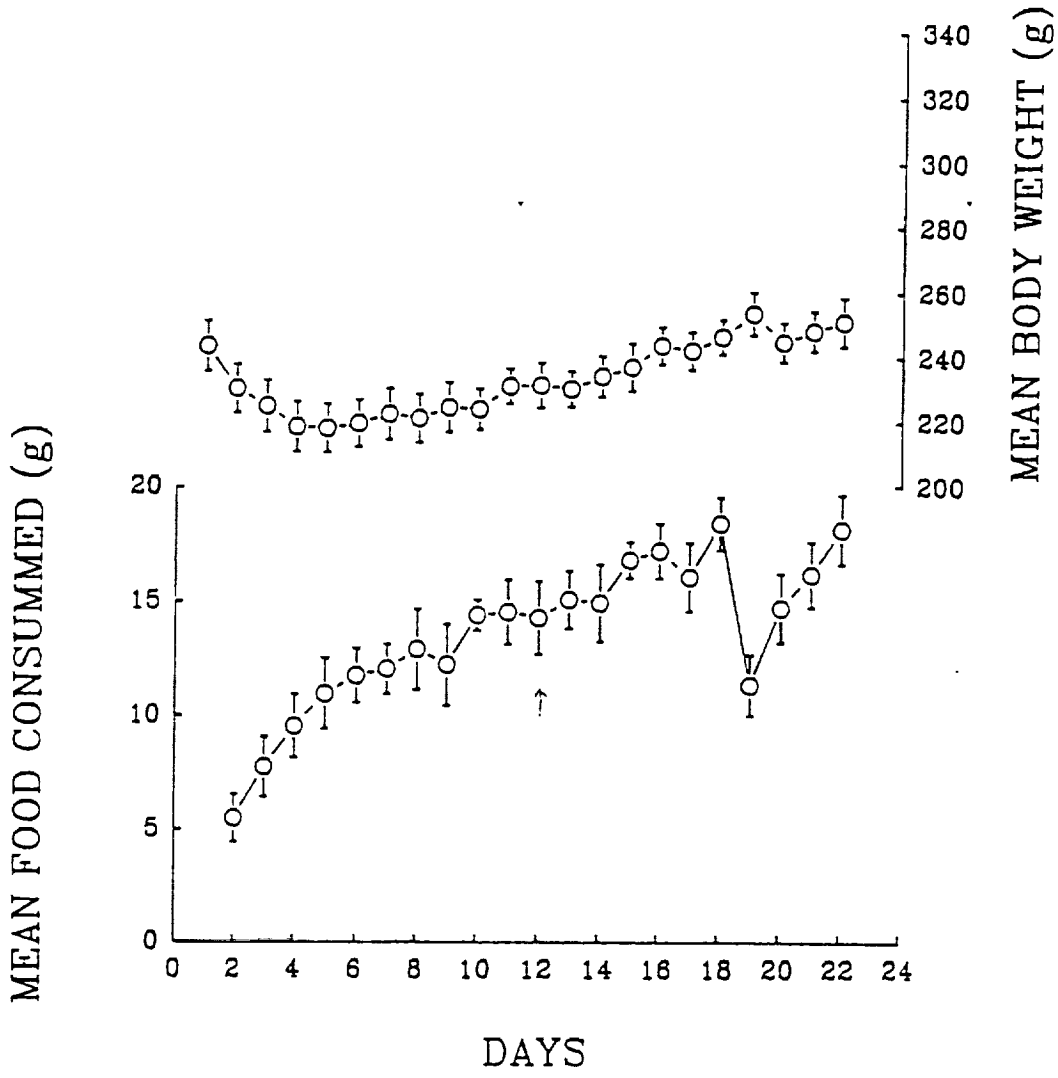

Figure 2. Mean Food Intake and Body Weight by Rats Exposed to Parabolic Flight.

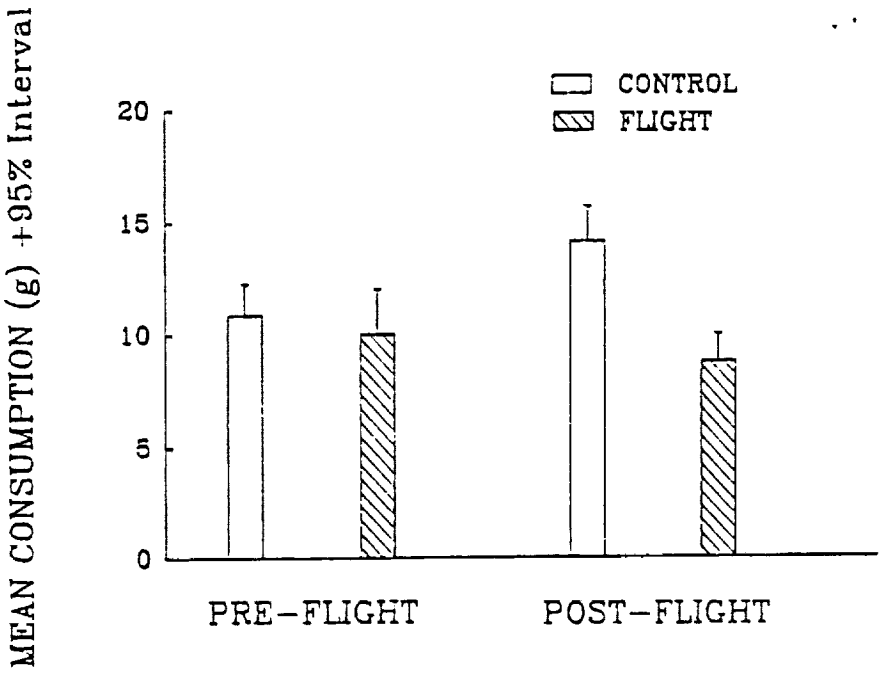

Figure 3. Mean Fluid Intake by Rats Prior to and Following Exposure to Parabolic Flight.

VI. APPENDIX I.

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VII. APPENDIX II.

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223.2 CHANGES IN PLASMA VASOPRESSIN DURING MOTION SICKNESS IN CATS. R. Fox*, L. Keil, N. Daunton, D. Thomsen*, M. Dictor*, and O. Chee*. NASA Ames Res. Center, Moffett Field, CA 94035 and San Jose State Univ., San Jose, CA 95192.

Changes in levels of plasma vasopressin (AVP) and cortisol (C) have been shown to be correlated with motion sickness and nausea in man. As part of the research aimed at validation of the cat as an appropriate animal model for motion sickness research, levels of these hormones were investigated in the cat during motion sickness elicited by vertical linear acceleration of approximately 0.6 Hz and 1 + 0.6 G.

In Study 1, 15 cats previously screened for susceptibility to motion sickness were prepared with indwelling jugular catheters to permit withdrawal of blood with minimal disruption of the stimulus and minimum stress to the animal. AVP and C were measured in blood samples obtained during exposure to vertical linear acceleration and during control sessions in which the animals were placed in the stationary apparatus. Samples were drawn according to a predetermined time schedule as follows: 10 min and 1 min prior to motion; 1, 5, 10, and 20 min after start of motion. Total duration of exposure to motion was 20 min. The data from this study indicate that both AVP and C are elevated during exposure to motion if emesis occurs. AVP reaches maximum levels during or about the same time as emesis, while C increases gradually throughout the period of vertical acceleration.

In Study 2, four cats were prepared with indwelling catheters and AVP was measured in blood withdrawn during exposure to the vertical linear acceleration. A single pre-motion sample was drawn 5 min prior to motion onset. Two series of samples consisting of three samples drawn at 3-min intervals were obtained during motion. The first series was initiated at emesis, and the second 25 min after emesis. Results show that levels of circulating AVP were elevated (2 to 27 times the control and pre-motion levels) in the samples taken during emesis and decreased, but remained 1 to 6 times above the pre-motion or control levels within 25 min.

The results of these two studies indicate that AVP is elevated during motion-produced emesis in the cat, and that AVP is more closely related to emesis than is C. These findings are in general agreement with those obtained from humans under motion sickness conditions, and indicate that it is appropriate to continue to use the cat in studies of hormone changes during motion sickness.

Conditioned Feeding Suppression in Rats Produced by Cross-Coupled and Simple Motions

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FOX, R. A., and N. G. DAUNTON. *Conditioned feeding suppression in rats produced by cross-coupled and simple motions.* Aviat. Space Environ. Med. 53(3):218-220, 1982.

Conditioned feeding suppression was produced in rats by earth-vertical rotation, seesaw acceleration, and cross-coupled accelerations. Rats were exposed to 15 min of motion immediately after eating a sweet, novel food. The effects of this motion were assessed by measuring consumption of sweet food during a second feeding session that was 72 hr after exposure to motion. Exposure to cross-coupled accelerations produced the greatest conditioned suppression of feeding. Progressively less suppression resulted from exposure to seesaw acceleration and to rotation. This ordering of suppression effects is consistent with the amount of vestibular stimulation produced by these motions. These results indicate that conditioned feeding suppression in rats can be produced by vestibular stimulation of a duration known to produce frank motion sickness in other animals and man.

ROTATION ABOUT AN EARTH-VERTICAL axis has been shown to reduce spontaneous activity (3) and drinking (7), to produce limited anorexia and pica (12,13), and to be an effective treatment for conditioned taste aversion (e.g., 1,5,10,12) in rats. Although the rat has an incomplete emetic system and does not vomit (8,16), it has been suggested that these effects result from "motion sickness" (1,3,5,7,10) and Mitchell and colleagues (12,13) have argued that pica should be considered a species-relevant measure of motion sickness in rats.

All of these studies have used rotation which is typically of higher speed (sometimes as high as 198 rpm) and longer duration (up to 2 h) than that known to produce motion sickness and emesis in man. More severe rotation parameters are, perhaps, used in animal studies because of the difficulty of producing vestibular stimulation which elicits any evidence of "sickness" in animals. In research with humans, volunteers are instructed to make standard head movements during rotation (4). These voluntary movements produce unusual, cross-coupled accelerative forces (2,6,9) on the vestibular system and are known to contribute to motion sickness. Animals typically are not required to move during rotation and such unusual forces on the vestibular system occur only during voluntary movements. The facts that spontaneous activity decreases in rats during rotation (3) and that squirrel monkeys which fail to reach frank motion sickness during rotation appear more likely to adopt a crouched, motionless posture than monkeys that vomit (14) imply that vestibular effects of rotation in animal studies are in part due to voluntary movement and are not specified by the experimenter.

Thus, while one approach to investigating motion sickness in animals has been to increase the speed and duration of rotation to insure that sickness occurs, a better approach would be to use a motion pattern that insures cross-coupled stimulation of the vestibular system independent of movements made by the animal. The present study evaluated the motion sickness response of rats using such a cross-coupled pattern with stimulus parameters similar to those used in human studies.

MATERIALS AND METHODS

Subjects: Long-Evans rats weighing 250 to 320 g were maintained in wire mesh cages located in an animal colony maintained on a 12:12 light:dark cycle. The 32 animals were assigned randomly to the four treatment

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Dr. Fox is with San Jose State University; and Dr. Daunton is with NASA-Ames Research Center.

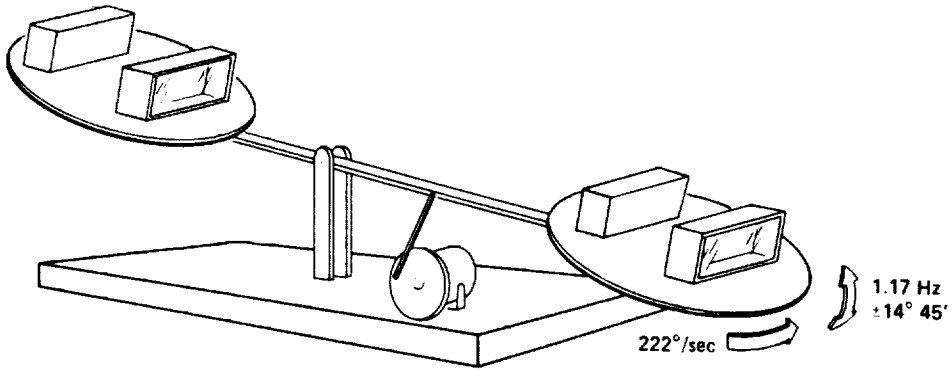

Fig. 1. A schematic illustration of the motion device illustrating the seesaw arm which produced vertical sinusoidal motion and the rotation disks with holding cages attached. For the complex motion condition seesaw action and rotation occurred simultaneously to produce cross-coupling of angular tilt with rotation.

groups (n = 8 per group). Water was available at all times and laboratory chow was ad lib. except during the 24 h immediately preceding the treatment and test periods.

Apparatus: Cross-coupled accelerations were produced by a device modeled after a motorized seesaw (Fig. 1). Aluminum disks were mounted directly on the shafts of two electric motors attached to opposite ends of a 220-cm aluminum arm with a center balance point. Two holding cages measuring 6.5 cm wide × 24.0 cm long × 12.8 cm high were mounted perpendicular to the radius of each disk with the centerline of each cage 20 cm from the axis of rotation. The disks rotated at 37 rpm (222 °/s). Vertical movement at the rate of 35 cpm (.58 Hz) was produced by an electric motor driving an off-center cam through a gear reduction box. This cam in turn drove a shaft connected to the seesaw arm, causing the ends of the arm to move up and down. The aluminum disks supporting the holding cages moved in arcs with radii of 110 cm and a vertical excursion of 56 cm.

Procedure: All animals were deprived of food for 24 h prior to the motion treatment. A novel, sweet food consisting of 9 g of chocolate candy was placed in each home cage for the hour preceding motion treatment. Consumption of this food was determined by weighing the food prior to and immediately following this period. Treated animals were then placed into the motion apparatus holding cages and either rotated around the earth-vertical axis, bounced (seesaw motion), or rotated and simultaneously bounced (cross-coupled motion) for 15 min. Animals in the control condition were placed into the holding cages on a stationary apparatus for 15 min. Following these treatments animals were returned to the colony where standard laboratory chow and water were available ad lib for 48 h. The effects of motion were assessed by measuring consumption of sweet food during a second feeding session that was 72 h after exposure to motion. Laboratory chow was removed 24 h prior to this session. During the test session approximately 9 g of the chocolate candy was again placed into the home cage for 1 h and the amount of this candy consumed was determined.

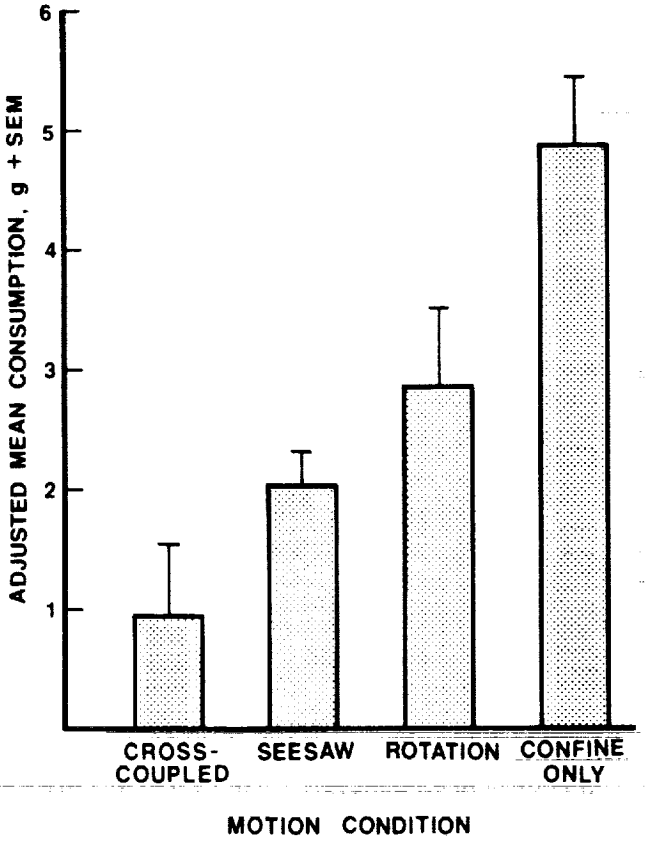

Fig. 2. Adjusted mean consumption of candy during the 1 hr test period which was 72 hr after motion treatment. The SEM is shown as a vertical bar.

RESULTS

The suppressive effects of the three motion conditions were assessed by examining the amount of candy consumed during the 1-h test session which occurred 72 h after the motion treatments. Differences between conditions were evaluated by an analysis of covariance, with amount of candy consumed prior to exposure to motion in the treatment session serving as a covariate to control for random variation between groups due to small sample size. Cross-coupled motion produced the most feeding suppression (Fig. 2), with progressively less suppression resulting from seesaw acceleration and rotation, $F(3,27) = 4.63$, $p<0.01$. Individual comparison tests between motion groups and the control indicate that feeding was suppressed in all motion conditions relative to the control, every $F(1,27) \geq 5.89$ and every $p<0.025$.

DISCUSSION

The observed ordering of the conditioned suppressive effects found in this study is consistent with the ordering of amount of vestibular stimulation resulting from these motions. Precise analysis of vestibular effects is not pos-

sible in an unrestrained animal because voluntary head movements during motion can produce cross-coupled accelerations. However, if such voluntary movement is ignored, the following general analysis applies to the conditions used here. The semicircular canals respond to angular acceleration and the otolith organs respond to linear accelerations. Thus, vertical movement primarily affects the otolith organs while rotation primarily affects the canals.

In the rotation condition used here, angular acceleration occurred when rotation began and deceleration occurred when rotation ended, but a constant angular velocity with no acceleration was present through the 15-min stimulation period. In the seesaw condition, a vertical linear acceleration occurred in a 1.17-Hz sinusoidal pattern. In addition, as the seesaw arm moved from the upper to the lower extremes of its arc, the holding cage platform tilted 14° 45′ from the horizontal, imparting a sinusoidal angular motion about a horizontal axis. Given these conditions and no movement by the animals, there should have been more stimulation to both the otolith organs and canals during seesaw motion than to the semicircular canals during rotation. The vestibular stimulation was greatest in the cross-coupled motion condition, where the accelerations of the seesaw motion were combined with rotation about an axis constantly tilting through a 14° arc on either side of earth-vertical.

The greater relative effectiveness of the cross-coupled motion and its associated whole-body, cross-coupling action is consistent with predictions from motion effects on humans and squirrel monkeys (11) and from the sensory conflict concepts (15). This finding implies that motion-induced conditioned feeding suppression in rats is produced by vestibular effects and suggests that the same range of unusual vestibular stimulation affects rats as affects other animals and humans. This outcome provides further support to the assertion that rats may become "motion sick" in spite of the observation that they do not vomit.

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198.1 SUSCEPTIBILITY OF THE SQUIRREL MONKEY TO SEVERAL DIFFERENT MOTION CONDITIONS. R.A. Fox*, N.G. Daunton and J. Coleman*. San Jose St. Univ. and NASA-Ames Research Center, Moffett Field, CA, 94035.

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The exact stimulus eliciting vomiting in animal studies of motion sickness is difficult to specify because the vestibular stimulation produced by many motion conditions is confounded by voluntary movements by the animals. This is an important problem because experiments with animal models of motion sickness can provide useful information about antimotion sickness drugs or the role of neural mechanisms, only when animals are exposed to the same motion stimuli in each experimental session.

A series of tests were conducted to determine the susceptibility of 15 adult squirrel monkeys to motion sickness in freely moving and restrained test conditions. Canal stimulation was varied by exposing the monkeys in freely moving conditions to varying degrees of angular velocity (60,90,120,150 deg/sec), and in restrained conditions to one angular velocity (150 deg/sec) and to cross-coupling effects of whole-body roll movements during rotation. Otolith stimulation was investigated by using sinusoidal vertical linear acceleration during free movement conditions, and off-vertical rotation and earth-horizontal (BBQ) rotation while restrained.

The percentage of freely moving animals vomiting during vertical axis rotation was 27, 93, 86, and 92 for the angular velocities of 60, 90, 120, and 150 deg/sec respectively. None of the monkeys vomited during vertical axis rotation or cross-coupled rotation when restrained. Otolith stimulation appears to be a less provocative stimulus for the squirrel monkey as the percentage of animals vomiting were 13, 0, and 7 for the conditions of free movement during oscillation, restraint during off-vertical and BBQ rotation respectively.

Motion sickness to the point of vomiting occurred regularly only in conditions where self-motion was possible. Such effects could occur because voluntary movement during motion augments vestibular effects by producing self-inflicted cross-coupling, but the failure to elicit vomiting with experimenter-produced cross-coupling argues against this interpretation. Alternatively, these results might imply that feedback from movement control mechanisms may play an important role in sensory conflict as suggested by Oman's sensory-motor conflict theory.

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RELATIONSHIP OF AREA POSTREMA TO THREE PUTATIVE MEASURES OF "MOTION SICKNESS" IN THE RAT. R. Sutton*, R. Fox, and N. Daunton. San Jose State Univ. and NASA Ames Res. Center, Moffett Field, CA, 94035.

Although the rat has an incomplete emetic reflex, several species-specific responses to motion have been proposed as measures of "motion sickness" in rats. The purpose of this study was to determine the dependence of these responses on one of several neural structures known to be essential to motion-induced vomiting in species with a complete emetic reflex.

The Area Postrema (AP) has been shown to play an important role in the production of motion sickness in vomiting species (Brizzee, et al., 1980; Wang and Chinn, 1954). In this study we compared the effects of thermo-cautery ablations of the AP on three different responses supposedly reflecting motion sickness in the rat: Conditioned taste aversion [CTA] (Braun and McIntosh, 1973); drinking suppression (Haroutunian, et al., 1976); and fecal boli (Ossenkopp, 1983). Efficacy of the ablations was determined by subjecting ablated, sham-operated, and unoperated control animals to a CTA test which is known to require a functional AP. Animals with AP ablations failed to form CTA when 0.15 M LiCl was paired with a 10% sucrose solution, while sham-operated control subjects conditioned as well as the unoperated control subjects. The extent of the ablations was evaluated histologically at the end of the experiment.

To determine the effects of the ablations on the measures of motion sickness, all animals were subjected to rotation for 30 min or 90 min on a platform displaced 20 deg from earth horizontal. Results indicate that ablation of AP in the rat has no effect on the formation of CTA to a 4% solution of cider paired with motion, on the suppression of drinking immediately after exposure to motion, or on the frequency of fecal boli during exposure to motion.

This failure of AP ablations to eliminate the effects of motion on any of these responses discourages their use as equivalents of motion-induced vomiting. The appropriateness of other suggested measures, e.g., pica (Mitchell, et al., 1976), remains untested, but the dependence of such measures on stimulation more severe than commonly used in motion sickness research and the absence of a demonstration of their dependence on neural structures essential to motion sickness in vomiting species, suggest caution in the use of such responses. Further, until more is known about the neural structures underlying these putative measures, the rat will remain a questionable subject in which to study motion sickness.

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MOTION SICKNESS: MECHANISMS, PREDICTION, PREVENTION AND TREATMENT

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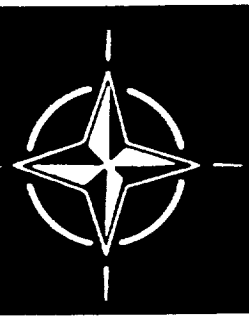

SUSCEPTIBILITY OF CAT AND SQUIRREL MONKEY TO MOTION SICKNESS INDUCED BY VISUAL STIMULATION: CORRELATION WITH SUSCEPTIBILITY TO VESTIBULAR STIMULATION

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SUMMARY

This paper describes experiments in which the susceptibility of both cats and squirrel monkeys to motion sickness induced by visual stimulation is documented. In addition, it is shown that in both species those individual subjects most highly susceptible to sickness induced by passive motion are also those most likely to become "motion" sick from visual (optokinetic) stimulation alone.

INTRODUCTION

It is well known that symptoms of motion sickness, as well as illusions of self-motion (circularvection and linearvection), can be elicited in human subjects by visual stimulation alone (1, 4, 5). Visual stimulation has also been shown to be effective in modifying the sickness-inducing effects of vestibular stimulation (2, 8). Further, in recent electrophysiological studies in animals it has been demonstrated that neural activity in the vestibular nuclei is modulated in a similar way by actual passive sinusoidal angular or linear acceleration of the animal and by visual stimulation which simulates those motions (3, 10). These findings suggest that vision should play an important role in the production of motion sickness in animal subjects as well as in human subjects, and, as in humans, the effects of visual stimulation should be greatest in those animals most susceptible to motion sickness produced by vestibular stimulation.

With the exception of the report of motion sickness in one squirrel monkey exposed to sinusoidal yaw-axis optokinetic stimulation (6), the susceptibility of animals to visually induced motion sickness has not been documented, nor has the relationship between susceptibility to motion sickness induced by visual vs. vestibular stimuli been addressed. The studies reported here were designed to investigate these factors in two species, the cat and the squirrel monkey. In these studies animals were subjected to passive acceleration provided by a two-pole swing (cats) or to passive rotation (monkeys), and to visual (optokinetic) stimulation which simulated these motions. Levels of susceptibility to visual stimulation alone were compared with those for the same animals exposed to the associated vestibular stimuli to obtain a better understanding of the role of vision in the production of motion sickness. The data were also analysed to determine how consistent the trait of susceptibility is across different stimulus conditions.

METHODS

Cats.

Twenty mature female cats were exposed to two conditions of visual and vestibular stimulation while free to move within a clear Plexiglas cage (44 cm X 16 cm X 21 cm). Animals were exposed to motion for a period of 20 min or until retching/vomiting plus 5 min, whichever period was longer. A period of not less than 30 days intervened between each of the tests.

Combined visual-vestibular stimulation was provided by a two-pole swing with a radius of 1.8 m, a frequency of 0.37 Hz, an arc of 1.0 rad, and a vertical displacement of 0.9 m. This swing was suspended within a large box-like enclosure, the interior of which was covered with patterned wallpaper and illuminated with a 100 watt bulb. A one-way vision port for observation of the animal was situated at one end of the box. The swing was manually pushed to provide the vestibular stimulation.

Visual stimulation alone was provided by the same two-pole swing and enclosure used in the combined stimulus condition, but in the Visual Only Condition the swing holding the cat remained stationary, while the enclosure was swung at a frequency of 0.28 Hz, with an arc of 1.0 rad. The visual stimulation was thus nearly, but not exactly, the same in the Combined Visual-Vestibular and the Visual Only Conditions. For the Visual Only Condition both observation ports were covered with one-way vision material.

Four additional motion sickness-inducing conditions involving visual-vestibular stimulation were used in an assessment of each subject's level of susceptibility to motion sickness. In Condition 1, a turntable was used to rotate the animals at 120 deg/sec. During rotation the cage holding the animal was tilted 7.5 deg above and below the horizontal plane at a frequency of 0.6 Hz. In Condition 2 the cage holding the animal was suspended from the end of a tilting beam which oscillated over a vertical distance of 2.1 m at 0.12 Hz. In Condition 3 the tilting beam was used to provide vertical oscillations at 0.42 Hz with a displacement of 1.0 m. A two-pole swing similar to that described for the Combined Visual-Vestibular Condition was used to provide the stimulus for Condition 4. In this condition the swing had a radius of 3.7 m, a frequency of 0.27 Hz, an arc of 1.5 rad, and a vertical displacement of 1.0 m. Both visual and vestibular stimulation were provided in these conditions, since the animals

could view the room through the Plexiglas cage during each of these tests. In all of these test situations retching/vomiting was detected by visual observation.

Monkeys.

Squirrel monkeys were exposed to the visual and vestibular stimulation while free to move in a clear Plexiglas cage (52 cm X 23 cm X 30 cm). Each test session lasted until 5 min after the time of retching/vomiting, or for a maximum of 30 min if vomiting did not occur. An interval of at least 30 days without testing was maintained between experimental sessions.

In the Combined Visual-Vestibular Condition the animals were rotated by a turntable (Goerz Model 611) while able to view the interior of the test room (a 2.3 m cube). The center of the turntable was located 1 m from the nearest corner on a room diagonal. In this condition the animals could see the observers and other contents of the test room, and therefore were exposed to very complex visual stimulation during rotation.

In the Visual Only Condition visual stimulation was provided by an optokinetic drum, the inside of which was covered with alternating white and dark green stripes, each subtending a visual angle of approximately 6.5 deg. In this condition the animal remained on the stationary turntable while the optokinetic drum rotated around the animal, providing optokinetic stimulation.

Two additional conditions (Vestibular Dark and Fixed Visual-Vestibular), studied extensively in another experiment (2), were used in the assessment of susceptibility to visual and vestibular stimulation. In the Vestibular Dark Condition the turntable holding the animal was rotated in the dark, and the animal received no visual stimulation. In the Fixed Visual-Vestibular Condition the optokinetic drum was coupled or fixed to the turntable and rotated with the animal, so that no optokinetic stimulation was produced by the rotation of the turntable.

Two different angular velocities were used to assess the effects of frequency of visual stimulation and amplitude of vestibular stimulation on relative provocativeness of the stimuli and on the correlation between susceptibility to visual vs. vestibular stimulation. Each monkey was exposed to each stimulus condition at both 60 and 150 deg/s. Motion sickness was assessed by determining latencies to retching/vomiting by audio monitoring.

RESULTS

Cats.

Two cats out of the 20 tested (10%) were made motion sick to the point of retching/vomiting under the Visual Only Condition. The latencies to the first retching/vomiting episode for the two cats were 2 min and 19 min. Five of the 20 animals (25%) were made motion sick by the combined visual-vestibular stimulation. The latencies to the first retching/vomiting episode ranged from 5 min to 17 min. The two animals which became sick in the Visual Only Condition were also made sick by the combined visual-vestibular stimulation. However, the remaining three animals which were susceptible to combined visual-vestibular stimulation were not made sick by visual stimulation. Thus, although neither stimulus produced high rates of sickness in these cats, of those animals which were made sick by combined visual-vestibular stimulation a subset was made sick when exposed to visual stimulation alone.

To determine whether there is a relationship between susceptibility to visual and to vestibular stimulation, two comparisons were made. One comparison involved determining whether the animals which retched/vomited to visual stimulation were those individuals with the shortest latencies to retching/vomiting in the condition involving combined visual-vestibular stimulation. If the animals which retched/vomited in the combined condition are ranked in order of ascending latencies, with a rank of 1 being the shortest latency (5 min) and a rank of 5 being the longest latency (17 min), it was found that the animals which retched/vomited to visual stimulation were not those ranked 1 and 2 on the combined stimulus, but rather those ranked 3 and 4. Thus, the two cats which retched/vomited to visual stimulation were not the most susceptible animals if latencies to retching/vomiting on the combined visual-vestibular test are used as the criterion of susceptiblility.

Another measure of susceptibility is available, however, since all of these animals had been tested for motion sickness in the four additional conditions involving rotation, vertical oscillation, and swinging. If animals are ranked on the basis of each of their responses in five repeated trials on each of the four additional motion sickness tests (a total of 20 test sessions), with Rank 1 assigned to the animal having the highest number of test sessions in which retching/vomiting occurred, then the two animals which vomited to visual stimulation were ranked 1 (vomited 10 times) and 2 (vomited 5 times) for susceptibility. By this measure, the animals made sick by optokinetic stimulation were indeed the most susceptible cats.

Monkeys.

The percentage of monkeys retching/vomiting in the Visual Only and Combined Visual-Vestibular Conditions and the median latency to the first sickness episode are shown in Table 1 for both angular velocities. The high percentage of animals vomiting to visual stimulation alone was quite unexpected on the basis of the data from the cat and from human studies, both of which typically show that susceptibility to visual stimulation is much lower than that to combined visual-vestibular stimulation. In this study the incidence of vomiting at the lower velocity was the same with visual and combined stimulation ($p > .50$), but the latency to sickness was shorter in the Visual Only Condition ($p < .01$). At the higher velocity, where greater vestibular effects would be expected, the incidence of sickness was greater ($p < .05$) and the latency to sickness was shorter in the Combined Visual-Vestibular Condition than in the Visual Only Condition ($p < .05$).

Table 3. Correlations Between Latencies to Retching/Vomiting (N = 27).

60 deg/s				
	VESTIBULAR DARK	COMBINED VISUAL-VESTIBULAR	FIXED VISUAL-VESTIBULAR	VISUAL ONLY
VESTIBULAR DARK	1.00	0.16	0.19	0.08
COMBINED VISUAL-VESTIBULAR	—	1.00	0.28	0.36
FIXED VISUAL-VESTIBUALR	—	—	1.00	0.53 *
150 deg/s				
	VESTIBULAR DARK	COMBINED VISUAL-VESTIBULAR	FIXED VISUAL-VESTIBULAR	VISUAL ONLY
VESTIBULAR DARK	1.00	0.20	0.34	0.13
COMBINED VISUAL-VESTIBULAR	—	1.00	0.74 *	0.56 *
FIXED VISUAL-VESTIBULAR	—	—	1.00	0.41

* $p < .05$

DISCUSSION

These studies indicate that in animal subjects, as in man, motion sickness can be elicited by visual stimulation alone, a condition which involves no direct stimulation of the vestibualar end organs by passive motion. This study has also shown that a subject's susceptibility to sickness induced by optokinetic stimulation is predictable from information about that subject's susceptibility to other motion conditions. In general these data indicate, as do data from studies with human subjects, that those individuals that are highly susceptible to motion sickness induced by passive motion are more likely to become sick, and/or become sick more rapidly, to visual stimulation alone, than are subjects that are relatively resistant to sickness induced by passive rotation.

While relative susceptibility in this population is predictable between some conditions, the determinants of this prediction are not clear. Current theories of motion sickness (7, 9) suggest that sickness-evoking properties of a situation depend upon, or evolve from, a complex interaction of stimulus characteristics and/or effects. Presumably these interactions among the visual, vestibular, and proprioceptive systems occur while the individual is maintaining postural and oculomotor control and performing goal-directed behaviors. The fact that, as shown above, correlations exist between different conditions at the two different velocities of stimulation implies that at the lower velocity, production of sickness in the Visual-Vestibular Fixed and Visual Only Conditions had some particular combination of visual-vestibular-proprioceptive factors in common. At the higher velocity the relationship among these factors was closer in the Combined Visual-Vestibular and Visual Only Conditions.

These data show that the particular combination of visual-vestibular-proprioceptive factors which produce motion sickness may be quite different under conditions of stimulation with similar motion components. It thus seems obvious that improved prediction of susceptibility to motion sickness will require extensive analysis of the specific components of motion stimulation which produce that sickness. In addition, these experiments have shown that both the cat and squirrel monkey, like man, are susceptible to motion sickness induced by visual stimulation alone. The fact that the squirrel monkey appears to be highly susceptible to visually-induced motion sickness suggests that this animal may be useful for more detailed analyses of the role of visual input in the production of motion sickness and for the assessment of parameters critical to successful prediction of susceptibility across sickness-inducing conditions.

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Table 1. Median Latency to Retching/Vomiting and Percentage of Animals Sick in Each of the Test Conditions (N = 27).

ANGULAR VELOCITY	MEASURE	VISUAL ONLY	COMBINED VISUAL-VESTIBULAR
60 deg/s	MDN. LATENCY (min)	10	25
	%-age SICK	74	70
150 deg/s	MDN. LATENCY (min)	11	8
	%-age SICK	81	100

To assess whether the highly susceptible animals were more likely to become sick when exposed to the optokinetic stimulus, those animals most and least susceptible to sickness in the Combined Visual-Vestibular Condition were selected as representative of the extremes of susceptibility induced by rotation. Animals with the 7 shortest and 7 longest (highest and lowest 25%) latencies from the extremes of the retching/vomiting latency distribution for each angular velocity were taken as representive of the least and most susceptible subjects for that velocity. The mean and median latencies to retching/vomiting to optokinetic stimulation at each angular velocity were then calculated for these highly susceptible and resistant animals. These data are shown in Table 2. Animals classified as susceptible to the combined visual-vestibular stimulation had shorter median latencies to retching/vomiting induced by optokinetic stimulation than did those classified as resistant. This relationship occurred for tests run at both 60 deg/s ($p = .04$) and 150 deg/s ($p = .03$) showing that animals susceptible to combined visual-vestibular stimulation were also those most susceptible to visual stimulation.

Table 2. Mean and Median Latency (min) to Retching/Vomiting Induced by Visual Stimulation at 60 and 150 deg/s for Animals Selected as Susceptible and Resistant on the Basis of Combined Visual-Vestibular Stimulation.

MEASURE	60 deg/s		150 deg/s	
	Resistant	Susceptible	Resistant	Susceptible
MEDIAN	26.0	7.3	22.0	4.7
MEAN	20.3	10.8	20.9	10.0

This conclusion is based upon an analysis of the relationship between the latencies to retching/vomiting for animals representing the extremes of the susceptibility spectrum, i.e., those which were either very susceptible or very resistant. Such an analysis could be misleading, since any relationship could depend predominately upon extreme ranges of susceptibilty, and thus might not reflect accurately the responses of the entire population of subjects.

To examine this issue further, we obtained correlations between sickness latencies from all monkeys across the four different sickness-inducing conditions, including Visual Only, Combined Visual-Vestibular, Vestibular Dark and Fixed Visual-Vestibular Conditions. The correlations between latencies obtained during visual stimulation alone and those obtained during the three other conditions of stimulation are shown in Table 3. These results indicate that at 60 deg/s (upper portion of the table) the level of sickness evoked in individual animals by the optokinetic stimulation is predicted better by the response of these animals to the Fixed Visual-Vestibular Condition than it is by the response to the Combined Visual-Vestibular or the Vestibular Dark Conditions. Conversely, sickness evoked by the optokinetic stimulus at 150 deg/s (lower portion of the table) is predicted better by the data obtained from the Combined Visual-Vestibular Condition than by those obtained in the other two conditions. Predictions about susceptibility to visual stimulation based on data obtained during vestibular dark stimulation, a condition not involving visual stimulation, is poor for both angular velocities.

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DISCUSSION

KENNEDY: Your prediction relationships are likely to be higher if you correct the correlations by the attenuation occasioned by the expected unreliability of the criterion. The latter may be best estimated by Guedry's data which suggest r =.50 is reasonable.

DAUNTON: The unreliability of the criterion (vomiting) is not due to unreliability of the measurement but is an inherent response unreliability. We regard the uncorrected correlations as good descriptors of the relationships among the variables reported here. On the other hand, it is of interest to know what the correlations might be without these unreliabilities, and we should examine such corrections.

OMAN: Do you have any data on test/retest reliability of any of the individual treatments (tests) you used? How does this compare with similar tests involving humans?

DAUNTON: As occurs with humans, some individuals respond on each exposure to motion while others respond on some tests but not others. As a general rule, we expect an analysis would show that animals are consistent in their responses on about 75% of the tests.

GUEDRY: Did you say that the animals were free to move within their container in all of the stimulus conditions you used?

DAUNTON: Yes, the animals move freely in the container-which was the same size in all of the conditions.

Appendix

REASSESSMENT OF AREA POSTREMA'S ROLE IN MOTION SICKNESS AND CONDITIONED TASTE AVERSION

N.G. Daunton*, K.R. Brizzee**, M. Corcoran*, G.H. Crampton***, F. D'Amelio*, S. Elfar**** and R.A. Fox****

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****Department of Psychology, San Jose State University, San Jose, CA 95192, U.S.A.

On the basis of classical studies on the role of the area postrema (AP) in motion-induced emesis it was generally accepted that the AP is an essential structure for the production of vomiting in response to motion (e.g., Wang and Chinn, 1954; Brizzee *et al.*, 1980). However, in more recent studies it has been demonstrated that vomiting induced by motion can still occur in animals in which the AP has been destroyed bilaterally (Borison, 1986; Corcoran *et al.*, 1985; Elfar *et al.*, 1986; Wilpizeski *et al.*, 1986). It has been inferred from some of these more recent studies that the AP plays no role in motion-induced emesis. From the standpoint of the current understanding of central nervous system (CNS) plasticity, redundancy, remodeling, unmasking, regeneration, and recovery of function, however, it is important to realize the limitations of using ablation procedures to determine the functional role of a given neural structure in a highly integrated, adaptable CNS. For example, the results of our recent investigations in cat and squirrel monkey on the role of the AP in emesis and conditioned taste aversion induced by motion indicate that while AP lesions do not prevent motion-induced emesis when animals are tested 30 days or more after surgery, the lesions do change the latency to emesis. Thus, contradictory findings from lesion studies must be evaluated not only in terms of species differences, differences in lesioning techniques and extent of lesions, and in stimulus parameters, but also in terms of duration of the recovery period, during which significant recovery of function may take place. In our judgment, inadequate consideration of the foregoing factors could lead to erroneous inferences about a given structure's role in the behavior of the intact, non-ablated animal.

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GAMMA-AMINOBUTYRIC ACID (GABA) AND NEUROPEPTIDES IN NEURAL AREAS MEDIATING MOTION-INDUCED EMESIS.

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In the present study, immunocytochemical methods were employed to localize the neurotransmitter amino acid gamma-aminobutyric acid and the neuropeptides substance P and Met-enkephalin in the area postrema (AP), area subpostrema (ASP), nucleus of the tractus solitarius (NTS), dorsal motor nucleus of the vagus nerve (DMNV) and lateral vestibular nucleus (LVN).

GABA: Glutamic acid decarboxylase immunoreactive (GAD-IR) terminals and fibers were observed in the AP and particularly in the ASP. A gradual decrease in the density of terminals was seen towards the solitary complex. The DMNV revealed irregularly scattered GAD-IR terminals within the neuropil or closely surrounding neuronal cell bodies. The LVN, particularly the dorsal division, showed numerous axon terminals which were mostly localized around large neurons and their proximal dendrites.

SUBSTANCE P: Substance P immunoreactive (SP-IR) terminals and fibers showed high density in the solitary complex, in particular within the lateral division. The ASP showed medium to low density of SP-IR fibers and terminals. The AP exhibited a small number of fibers and terminals irregularly distributed. The DMNV revealed a high density of SP-IR terminals and fibers that were mainly concentrated in the periphery. Very few terminals were detected in the LVN.

MET-ENKEPHALIN: Met-enkephalin immunoreactive (Met-Enk-IR) fibers and terminals showed high density and uniform distribution in the DMNV. Scattered terminals and fibers were observed in the AP, ASP and NTS (particularly the lateral division). The very few fibers and terminals observed in the LVN surrounded the neuronal cell bodies.

The present report is part of a study designed to investigate the interaction between neuropeptides and conventional neurotransmitters under conditions producing motion sickness and in the process of sensory-motor adaptation.

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186.2 DETECTION OF EMETIC ACTIVITY IN THE CAT BY MONITORING VENOUS PRESSURE AND AUDIO SIGNALS. A. Nagahara*, R. Fox, M.Daunton, S. Elfar*. San Jose State University and NASA Ames Research Center, Moffett Field, CA, 94035.

To investigate the use of audio signals as a simple, noninvasive measure of emetic activity, we studied the relationship between the somatic events and sounds associated with retching and vomiting. Thoracic venous pressure obtained from an implanted external jugular catheter has been shown to provide a precise measure of the somatic events associated with retching and vomiting (McCarthy & Borison, 1974). We compared changes in thoracic venous pressure monitored through an indwelling external jugular catheter with audio signals, obtained from a microphone located above the animal in a test chamber. In addition, two independent observers visually monitored emetic episodes. Retching and vomiting were induced by injection of xylazine (0.66mg/kg s.c.), or by motion.

A unique audio signal at a frequency of approximately 250 Hz is produced at the time of the negative thoracic venous pressure change associated with retching. Sounds with higher frequencies (around 2500 Hz) occur in conjunction with the positive pressure changes associated with vomiting. These specific signals could be discriminated reliably by individuals reviewing the audio recordings of the sessions.

Retching and those emetic episodes associated with positive venous pressure changes were detected accurately by audio monitoring, with 90% of retches and 100% of emetic episodes correctly identified. Retching was detected more accurately ($p<.05$) by audio monitoring than by direct visual observation. However, with visual observation we were able to identify a few incidents in which stomach contents were expelled in the absence of positive pressure changes or detectable sounds. These data suggest that in emetic situations, the expulsion of stomach contents may be accomplished by more than one neuromuscular system and that audio signals can be used to detect emetic episodes associated with thoracic venous pressure changes.

Neuroscience Abstracts, Volume 12 Part 2, November 1986

242.13 RECOVERY OF THE VOMITING REFLEX FOLLOWING AREA POSTREMA ABLATION IN SQUIRREL MONKEYS. S. Elfar, N. Brizzee, R. Fox, M. Corcoran, N. Daunton, J. Coleman*. San Jose State Univ., San Jose CA, Delta Regional Primate Center, Covington LA, and NASA Ames Research Center, Moffett Field CA.

The role of the area postrema (AP) in motion-induced emesis has been re-assessed recently in several different species (e.g. Borison et al., 1984; Corcoran et al., 1985; Wilpizeski, 1986). In a few of these studies, the role of the AP in motion-induced conditioned taste aversion (CTA) has also been addressed (e.g. Sutton et al., 1983; Corcoran et al., 1985). The purpose of the present study was to extend this comparative study to the squirrel monkey, to evaluate further the role of AP in vomiting, and to investigate the dynamics of the recovery process.

The AP was ablated bilaterally in 7 motion-susceptible squirrel monkeys which previously had been characterized in terms of their responses to various motion sickness-inducing stimuli. After recovery from surgery all animals were tested at 30-day intervals for a period of 11 months to determine the effects of AP ablations on susceptibility to the same sickness-inducing conditions. In addition, the effectiveness of motion in producing CTA was evaluated. All pre-ablation motion tests involved stimulation for 30 min., while post-lesion tests were 60 min. in duration.

All animals showed significant increases in latencies to vomiting after AP ablations. However, the latencies tended to decrease with time after the ablation. All but one animal vomited on at least one of the 10 motion tests occurring after ablation of AP. In addition, CTA was produced by motion in animals which continued to vomit after AP ablation, whether or not vomiting occurred during the 30 min. of motion used in the conditioning sessions. These results suggest that structures other than AP, and processes other than those mediated through AP, may play an important role in motion-induced emesis.

FORE-AFT SINUSOIDAL MOVEMENT		VIS-VSTB FIXED	VIS-VSTB CONC.	VIS ONLY	VSTB DARK
PIGEON POSTURAL SWAY (AMPLITUDE)	MEAN	21	13	8	20
	SEM	1.8	1.1	1.4	1.5
CAT NEURAL UNIT RESPONSE (GAIN)	MEAN	118	151	79	119
	SEM	19.2	19.4	11.3	20.2

Fig. 2. Upper section: Schematic diagram of visual and vestibular stimulus conditions for fore-aft linear motion. Inner rectangle represents platform on which the animal cage is placed and which provides the vestibular stimulation. Outer square represents visual stimulus surrounding platform. Arrows indicate which device(s) is (are) moved in each condition. *Middle section:* Means and standard errors of postural sway amplitude from 10 pigeons each subjected to 10 cycles of translational motion under each stimulus condition. *Lower section:* Mean and standard error of gain of 21 vestibular nuclear units in the cat.

The effects of these confirming and conflicting VV stimuli on single unit activity in the vestibular nuclei of the cat during fore-aft translational movement are also shown in figure 2. The gain of the response is suppressed under the fixed VV condition as compared with that obtained under the concordant VV condition. Similar effects on vestibular unit activity have been shown in the Rhesus subjected to rotation [8]. These results are consistent with the effects of confirming and conflicting VV stimuli on the EMG from leg muscles of humans and baboons [1].

These studies show that the fixed VV condition produces a decrease in the gain of vestibular unit responses and of the EMG which appear to be associated with deterioration in the control of posture and increased susceptibility to motion sickness. When the incidence of sickness from the present experiment using freely moving animals is compared with that using fully restrained animals [4], there is little doubt that the provocativeness of VV stimulation is modulated by postural effects. Thus, it appears that the relationship between visual, vestibular, and postural control mechanisms may be an important one that should not be ignored in future studies of motion sickness. We suggest that the methods used to

assess the contributions of visual, vestibular, and proprioceptive inputs to postural control could be used productively to study the sensory and motor components underlying motion sickness.

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YAW-AXIS ROTATION	VIS-VSTB FIXED	VIS-VSTB CONC.	VIS ONLY	VSTB DARK
SURROUND	60°/s	-	60°/s	-
TABLE	60°/s	60°/s	-	60°/s
% SICK	95	77	82	45
MDN. LAT.	3.6	8.0	9.3	30.0
SURROUND	150°/s	-	150°/s	-
TABLE	150°/s	150°/s	-	150°/s
% SICK	100	95	75	90
MDN. LAT.	3.0	6.0	14.5	11.0

Fig. 1. Upper section: Schematic diagram of visual and vestibular stimulus conditions for vertical axis rotation. Rectangle represents animal cage on turntable (inner circle). Outer circle represents optokinetic drum. Arrows indicate which device(s) is (are) rotated in each condition. *Lower section:* Percentage of animals retching/vomiting and median latency to first retching/vomiting episode for subjects exposed to each condition of visual and vestibular stimulation at 60°/s (n = 22) and at 150°/s (n = 20).

Results

The data on percentage of animals retching/vomiting and the median latency to the first retching/vomiting episode are shown in figure 1. The high percentage of animals vomiting under the visual only condition was quite unexpected, since other studies dealing with visually evoked motion sickness in animals have yielded percentages much lower than those found with vestibular stimulation alone. In the present study, the incidence of vomiting at the lower velocity was almost twice as great with visual stimulation as with vestibular stimulation. At the higher velocity, where greater vestibular effects would be expected, the vestibular stimulation was slightly more provocative than the visual stimulation in terms of median latency to vomiting ($p < 0.05$), and marginally more provocative in terms of the percentage of animals made sick ($p < 0.10$). The fact that the visual stimulation was more provocative at the lower velocity is expected from studies which show that the visual system provides maxi-

mal information about self-motion at low frequencies of stimulation, while the vestibular system provides the majority of information about self-motion at high frequencies [9].

When comparing the effects of the two combined VV stimulus conditions, figure 1 shows that the inconsistent (fixed) VV condition yields higher sickness rates and shorter latencies to vomiting than the consistent VV condition. The differences between percentages of animals vomiting were significant at 60°/s ($p < 0.05$), but not at 150°/s ($p > 0.30$). The differences between median latencies to vomiting were significant at both angular velocities ($p \leq 0.01$).

Discussion

The results of this experiment confirm the findings from studies of the effects of different VV conditions on motion sickness induced in human subjects by rotation [2], and of the effects of different visual and vestibular conditions on motion sickness in restrained squirrel monkeys subjected to sinusoidal angular acceleration [4]. In these studies, as well as in the present study, conflicting VV stimulation has been shown to be more provocative than consistent or confirming VV stimulation.

Ideally, to confirm that a relationship exists between the effects of different visual and vestibular stimuli and the theory that motion sickness occurs under conditions in which control of movement is disrupted, we would like to have had measures of postural reflexes to assess the amount of disruption under several VV conditions. While our future plans involve the simultaneous collection of such postural and motion sickness data, some data available from our own studies and from those of others do suggest that motion sickness is greatest under conditions in which postural control is maximally disrupted.

The results of two experiments in which the fixed and concordant VV conditions were used to evaluate postural sway and activity in the vestibular nuclei are shown in figure 2. In these experiments the stimulus was fore-aft translational motion (0.15 *G*; 0.59 Hz). A comparison of the amplitude of the pigeon's body sway in the two conditions shows that there was greater disruption of postural control (greater sway amplitude) in the fixed than in the concordant VV condition. Similar effects of confirming and conflicting VV stimulation on postural control have been shown in the dog [7] and in humans [5].

Igarashi, Black (eds.), Vestibular and Visual Control on Posture and Locomotor Equilibrium. 7th Int. Symp. Int. Soc. Posturography, Houston, Tex., 1983, pp. 164–169 (Karger, Basel 1985)

Motion Sickness Elicited by Passive Rotation in Squirrel Monkeys

Modification by Consistent and Inconsistent Visual Stimulation[1]

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Introduction

Current theory and recent evidence suggest that motion sickness occurs under conditions of sensory input in which the normal motor programs for producing eye, head, and body movements are not functionally effective, i.e. under conditions in which there are difficulties in maintaining posture and controlling eye movements [6]. Conditions involving conflicting or inconsistent visual-vestibular (VV) stimulation should thus result in greater sickness rates since the existing motor programs do not produce effective control of eye-head-body movements under such conditions.

We feel that the relationship of postural control to motion sickness is an important one and one often overlooked. We reported the results of a study which showed that when postural requirements were minimized by fully restraining squirrel monkeys during hypogravity parabolic flight, no animals became motion sick, but over 80% of the same 11 animals became sick if they were unrestrained and maintained control of their posture [3].

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On the basis of these results it appears that postural requirements may modulate the effects of visual and vestibular inputs on the production of motion sickness. For this reason it would seem appropriate to investigate motion sickness under experimental conditions similar to those used in studies of postural control so that the contributions of visual, vestibular, and proprioceptive inputs to the development of motion sickness, as well as to reflex responses, can be determined in an orderly manner. As the first step in our research using this approach, we have investigated the effects of visual stimulation on motion sickness susceptibility of the freely moving squirrel monkey.

Methods

Squirrel monkeys were exposed to each of four different conditions of visual and vestibular stimulation while free to move in a clear Plexiglass cage (52 × 23 × 30 cm). Each session lasted until 5 min after the time of vomiting or for a maximum of 30 min if vomiting did not occur. An interval of at least 30 days without testing was maintained between experimental sessions.

An optokinetic drum and turntable provided the visual and vestibular stimulation. The optokinetic drum was covered with alternating white and dark green stripes, each of which subtended a visual angle of approximately 6.5°. The turntable (Goerz Model 611) and drum could be rotated separately or together.

The four visual and vestibular conditions of stimulation used in this experiment are shown schematically in figure 1. Two baseline conditions were used to determine the separate effects of vestibular stimulation and visual stimulation on motion sickness responses. In the vestibular dark condition the turntable holding the animal was rotated in the dark, and the animal received no visual stimulation. In the visual only condition, the turntable remained stationary, while the optokinetic drum rotated around the animal, providing optokinetic stimulation.

The two conditions of greatest interest in this study involved combined VV stimulation. In the concordant VV condition the turntable was rotated within the drum which was stationary with respect to the room (i.e. the normal condition that occurs whenever an organism moves within an earth-fixed environment). Consistent visual and vestibular cues were provided in this condition. In the fixed VV condition, the optokinetic drum was coupled or fixed to the turntable and rotated with the animal, so that no optokinetic stimulation was produced by the rotation. This arrangement results in inconsistent or conflicting VV stimulation.

Under each of the conditions of visual and vestibular stimulation, two different angular velocities were tested, 60°/s ($n = 22$) and 150°/s ($n = 20$). In all conditions postural requirements, and indirectly, vestibular stimulation, were dependent upon the characteristics of free movement produced by each animal within the testing cage. Motion sickness was assessed by determining latencies to retching and/or vomiting by audio monitoring.

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AREA POSTREMA ABLATIONS IN CATS: EVIDENCE FOR SEPARATE NEURAL ROUTES FOR MOTION- AND XYLAZINE-INDUCED CTA AND EMESIS.

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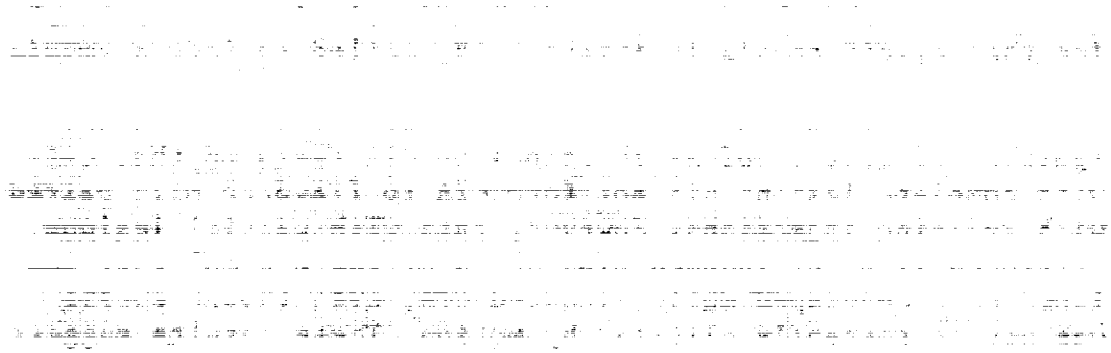
Previous studies on the role of the area postrema (AP) in vomiting induced in the cat by motion and drugs have shown that the AP is not essential for motion-induced vomiting, but is necessary for vomiting to apomorphine and xylazine. To confirm these findings and to determine the role of the AP in the formation of Conditioned Taste Aversion (CTA), the AP was ablated bilaterally in 10 adult female cats. With one exception, the ablated cats continued to vomit to the same motion that elicited emesis before the ablation. Doses of xylazine and apomorphine that elicit emesis in intact cats, failed to induce emesis in the ablated cats. Histological examination indicated that 8 cats had complete lesions and 2 had partial lesions. Investigations of effects of AP ablations on CTA revealed that cats with complete lesions did not form CTA to flavored milk paired with xylazine injections. However, cats with partial lesions developed xylazine-induced CTA. Seven of the 8 completely lesioned cats developed motion-induced CTA, even though emesis was not consistently elicited by motion. These results suggest that there are multiple routes for inducing CTA and the emetic reflex, that CTA can form without eliciting emesis, and that CTA may be a sensitive measure of sub-emetic motion sickness.



Off-Vertical Rotation Produces Conditioned Taste Aversion and Suppressed Drinking in Mice

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FOX RA, LAUBER AH, DAUNTON NG, PHILLIPS M, and DIAZ L. *Off-Vertical rotation produces conditioned taste aversion and suppressed drinking in mice.* Aviat. Space Environ. Med. 1984;55:632–5.

The effects of off-vertical rotation upon the intake of tap water immediately after rotation, and upon conditioned taste aversion, were assessed in mice with the tilt of the rotation axis varying from 5 to 20° from the earth-vertical. Conditioned taste aversion occurred in all mice that were rotated, but the intake of tap water was suppressed only in mice that were rotated at 15 or 20° of tilt. The greater suppression of tap water intake and the stronger conditioned aversion in the mouse as the angle of tilt was increased in this experiment are consistent with predictions from similar experiments with human subjects where motion sickness develops more rapidly as the angle of tilt is increased. It was suggested that off-vertical rotation may be a useful procedure for insuring experimental control over vestibular stimulation in animal studies of motion sickness.

MOST STUDIES OF MOTION sickness involving animal subjects use procedures in which the animals are permitted some voluntary movement during exposure to the sickness-inducing motion. In fact, voluntary motion is necessary to obtain motion sickness in human and animals subjects during vertical axis rotation if the subjects are located on or close to the axis of rotation (10). Methods for insuring that vestibular stimulation will result from rotation even if voluntary movement does not occur or is reduced have involved combining sinusoidal vertical accelerations with vertical axis rotation (12), moving a rotating disk through an arc on the arm of a seesaw (2), alternating 15-s periods of rotation with 5-s periods of no rotation (6,11), and using sinusoidal yaw axis rotation (7). All of these methods involve complex vestibular stimuli which are often difficult to quantify and to generate. A simple method for producing specified vestibular stimulation which makes animals motion sick in the absence of voluntary movement could be useful for future studies.

It is known that motion sickness can be induced readily in human subjects producing no voluntary movement if rotation about an axis displaced from earth-vertical is used as the eliciting stimulus (3,8). The effects of various degrees of off-vertical rotation have not been evaluated previously in animal studies. The experiment reported here was conducted as a peliminary examination of the usefulness of this stimulation as a method of generating controlled vestibular stimulation in animals. Mice were used as subjects in this study and conditioned taste aversion and drinking suppression were used as measures of the effects of vestibular stimulation.

METHODS

Subjects: The mice, 72 male Swiss-Webster weighing 20–23 g, were housed 6 per cage and maintained on a 12:12 light:dark cycle. Animals were assigned randomly to one of six treatment groups with 12 mice in each group.

Apparatus: The off-vertical rotation device consisted of an aluminum disk mounted directly on the shaft of a gear reduction box driven by a Bodine motor. This disk

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rotated clockwise at 204°·s^{-1} (34 rpm). Holding cages were constructed by placing two plexiglass dividers into aluminum chassis boxes (6.5 cm wide × 24.0 cm long × 12.8 cm high) to form three compartments (6.5 cm × 8.0 cm × 12.8 cm) in each chassis box. Four such boxes were mounted perpendicular to the four radii of the disk, 12 cm from the axis of rotation. Thus, each mouse was positioned 12.0 to 15.0 cm from the axis of rotation. With the angular velocity of 204°·s^{-1}, forces of 0.16 and 0.19 G occurred at 12.0 and 15.0 cm distances from the axis of rotation. Tilt was accomplished by elevating one end of the device to produce tilts of the axis of rotation of 0, 5, 10, 15 and 20° from the earth vertical. The vertical excursion resulting from off-vertical rotation produced an additional force of up to +0.05 G at the tilt angle of 20° with lesser forces at the smaller angles of tilt.

Procedure: During the first 6 d of the experiment the mice were adapted to a restricted drinking regimen. Each d the mice were placed into individual cages and allowed to drink from a 20-ml pipette inserted into each cage. The animals had access to tap water for 10 min. Water was then removed for 15 min (a rest period). After this period the animals were given access to tap water and food in the individual cages for an additional 20 min. Thus, the mice had access to water for 30 min (10 min plus 20 min) each d. The intake of fluid during the two drinking periods was determined from pipette readings.

On Day 7 mice were offered a sweet flavored solution (2 g saccharin·L^{-1} of tap water) during the initial 10-min drinking period. This drinking period was followed immediately by exposure to rotation for 15 min at one of the five angles of tilt, or, for the control group, by confinement in a stationary apparatus for 15 min. The next 2 d were for recovery and the same drinking regimen used during the adaptation period (tap water in each drinking period) was repeated. On Day 10 the mice were again offered the flavored solution during the first 10-min drinking period, which was followed by the 15-min rest period, and finally by the 20-min period in which access to both food and tap water was given.

RESULTS

The effects of angle of tilt were assessed using suppression of drinking following rotation and conditioned taste aversion as measures. General suppression of drinking produced by rotation was determined by measuring the intake of tap water immediately after rotation. The average intake of fluid by each group is shown in Fig. 1. There was no difference between controls and rotated animals in the intake of tap water during the 20-min drinking period on the day preceding the rotation [$F(5,66)<1$]. However, immediately after rotation, the intake of tap water decreased as the angle of tilt increased [$F(5,66)=2.70$; $p<0.05$]. The relationship between intake of tap water and angle of tilt was examined further using Dunnett's Test to compare results from all treatment groups with those from the control group. This test indicated that after rotation, intake of tap water was suppressed for tilt angles of 15 and 20° ($ps<0.05$) but that the response of the groups exposed

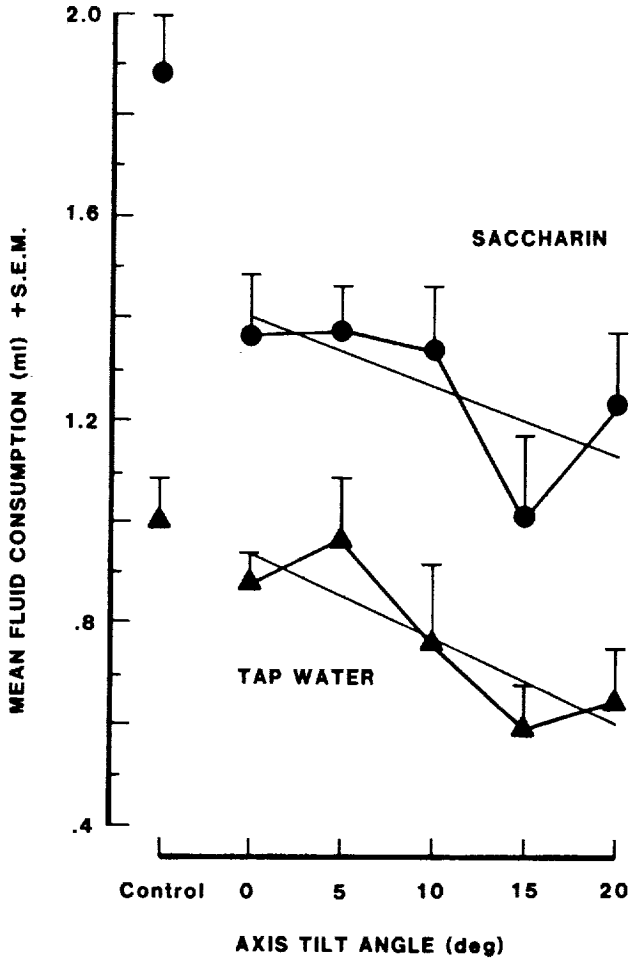

Fig. 1. Average consumption of saccharin water (3 d after conditioning) and of tap water (immediately after rotation) by the control subjects and by the animals rotated at various angles of tilt from earth-vertical.

to other angles of tilt did not differ from that of the control group ($ps>0.05$).

The conditioned taste aversion produced by varying angles of tilt was assessed by determining differences in the consumption of saccharin flavored water 3 d after the rotation. Prior to rotation the control and rotation groups did not differ in the amount of saccharin water drunk [$F(5,66)=1.33$, $p>0.25$]. Following the rotation, however, conditioned taste aversion was present in all treatment groups [$F(5,66)=5.06$, $p<0.005$]. Comparison of results from all treatment groups with those from the control group using Dunnett's test, reflected a lower intake of saccharin water by animals that were rotated than by the non-rotated control animals ($p<0.05$ for tilt angles of 0 and 5° and $ps<0.01$ for tilt angles of 10, 15 and 20°).

The linear correlation of the average intake of fluid of each treatment group with the angles of tilt of 0–20° was determined for both tap water (suppression of drinking measures, $r=-0.89$) and saccharin flavored water (taste aversion measure, $r=-0.63$). This analysis indicates that the tap water variable used to assess the suppression of drinking reflects a stronger linear relationship with the angle of tilt than does the conditioned taste aversion measure (i.e., saccharin intake). In addition, the regression line for tap water predicts intake

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by the control animals, which were not rotated, while the regression line for the saccharin variable does not predict intake by the saccharin control animals.

DISCUSSION

The results of this study show that off-vertical rotation produces effects on conditioned taste aversion and drinking suppression—two putative measures of motion sickness (1,5,6,9)—which would be predicted from human studies of motion sickness using off-vertical rotation. Miller and Graybiel (8) have reported a close linear relationship between the logarithmically scaled off-vertical angle of tilt and the time to evoke malaise IIA in man with tilt angles of 7.5–25° (see [4] for a description of the sickness rating scale). To compare the results of this study with those of Miller and Graybiel, the correlations of angles of tilt with each of the measures of "sickness" was determined for the range from 0–20° of tilt (see Fig. 1). As the angle of tilt increases, both measures reflect the increasing effects of rotation in mice as in man.

When all of the groups that were rotated at the various angles of tilt were compared with the non-rotated control group, the effects of tilting were found to be different for the two measures. As reflected by the conditioned taste aversion measure, intake of the saccharin solution was suppressed in all groups that were rotated. On the other hand, if suppression of tap water intake immediately after rotation was used as the measure, significant effects were found only for the two groups rotated at the two greatest angles of tilt (15 and 20°), i.e., where the vestibular stimulation was greatest. These results indicate that conditioned taste aversion is a more sensitive measure of the aversion effects of vestibular stimulation than is suppression of the intake of a familiar fluid immediately after rotation. This result also implies that the taste aversion paradigm might be preferred if treatment effects are expected to be small. It should be kept in mind, however, that the sensitivity of this measure might result in "floor effects" if motion parameters are severe. On the other hand, the measurement of water intake is obtained more easily than the measurement of taste aversion and might be considered the preferred measure of the effects of motion because of the continuous relationship observed between intake of water and angle of tilt in this experiment.

In discussing the measure involving post-rotary intake of tap water, is should be noted that with the drinking regimen used in this experiment the suppression of this intake might be affected by transient effects of the motion (e.g., ataxia from vestibular stimulation) rather than by motion-induced "sickness" per se. Two observations indicate that transient disruptive effects are not the sole cause of the suppression of drinking. First, the animals were able to drink immediately following rotation as evidenced by the fact that they often drank a small amount of water immediately on being placed into the drinking area. Second, no quantifiable ataxia or other motor disruption was observed during the drinking sessions. In addition, it was possible to compare the intake of water during each half of the 20-min drinking period which followed the motion treatment. If drinking were suppressed by disruption of motor responses, intake might be expected to increase with recovery after rotation. In fact, the opposite occurred on treatment days with motion and as well as on control days with no motion. While these observations might suggest that intake of water after rotation reflects "sickness" rather than inability to drink due to disruption of motor control, such an interpretation is not required, and it must remain a subset of the larger question of how to interpret "motion sickness" in species which do not have a complete emetic reflex.

This same argument regarding transient disruption of motor systems does not apply to conditioned taste aversion because the magnitude of this aversion was assessed in a test made 3 d after the motion treatment. In spite of this 3-d recovery period, conditioned aversion appears to be a more sensitive measure of the effects of off-vertical rotation than the measure involving intake of tap water immediately after rotation. Further evidence for the sensitivity of conditioned taste aversion can be found in the demonstration that squirrel monkeys form conditioned aversions under motion sickness-evoking conditions even when emesis does not occur (12). It is not known whether the neural mechanisms involved in the formation of conditioned aversions are the same as those which lead to emesis, but the fact that conditioned taste aversions do develop without emesis suggets that such aversions could provide a sensitive measure of pre-emetic symptoms of motion sickness (e.g., nausea).

The effects of angle of tilt as reflected by conditioned aversion and drinking suppression in the mouse show that the mouse is affected by this stimulation in a manner that is predictable from studies of motion sickness in humans. This finding provides support for the use of off-vertical rotation in studies of motion sickness in animals since the stimulus to the vestibular apparatus can be specified quite precisely and varied easily when the voluntary movement of the animals is inhibited. Although there may be some question as to whether off-vertical stimulation is specific to the otolithic receptors or whether it also produces some canal stimulation (3), it is clear that off-vertical rotation of restrained animals would result in a much more controlled stimulation of the vestibular apparatus than is found in the typical animal study of motion sickness in which voluntary movements are neither controlled nor measured and voluntary movements by the animals might result in unspecified and undesirable cross-coupled accelerations.

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All procedures used in this project complied with the Guideline Principles in the Care and Use of Animals.

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Vasopressin and Motion Sickness In Cats

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Fox RA, Keil LC, Daunton NG, Crampton GH, Lucot J. *Vasopressin and motion sickness in cats.* Aviat. Space Environ. Med. 1987; 58(9, Suppl.):A143–7.

Levels of arginine vasopressin (AVP) in blood plasma and cerebrospinal fluid (CSF) were measured in cats under several motion-sickness-inducing conditions. Plasma AVP increased significantly in both susceptible and resistant animals exposed to motion. When vomiting occurred, levels of plasma AVP were dramatically elevated (up to 27 times resting levels). There was no difference in resting levels of AVP of susceptible and resistant cats. Levels of CSF-AVP were not elevated immediately after vomiting, but the resting levels of CSF-AVP were lower in animals that vomited during motion than in those animals which did not vomit during motion. The results of these experiments show that changes in systemic AVP are directly related to vomiting induced by motion, however, CSF-AVP apparently does not change in association with vomiting. CSF-AVP does appear to be lower in animals that reach frank vomiting during motion stimulation than in animals which do not vomit.

IT HAS BEEN demonstrated in humans that motion sickness reduces free water clearance in water-loaded individuals. This finding led to the conclusion that plasma arginine vasopressin (AVP) increased during motion sickness (11). Eversman *et al.* (3) measured several hormones in plasma by radioimmunoassay, and documented the elevation of growth hormone, prolactin, AVP, and cortisol following experimentally-induced motion sickness in man. These authors concluded that secretion of AVP was the most selective indicator for motion sickness.

In the experiments reported here, AVP was measured during motion sickness in cats in order to expand our knowledge of how this hormone might be related to motion sickness. In the first two experiments plasma cortisol and AVP were measured in each blood sample to evaluate whether AVP is related specifically to the emetic response or whether it is more closely related to nonspecific stress as reflected by cortisol. In the third experiment the level of AVP was measured in cerebrospinal fluid (CSF) during motion sickness to investigate whether central nervous system concentrations of AVP might play a role in the central stimulation of vomiting.

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EXPERIMENT I: CHANGES IN AVP DURING MOTION IN SUSCEPTIBLE AND RESISTANT CATS

There were two principal objectives of this experiment. One objective was to evaluate whether the magnitude of AVP secretion during motion sickness in the cat is related to the severity of the sickness, as it is in man (3). The second objective was to determine, in the cat, whether the resting level of AVP could serve as an index of individual susceptibility to motion sickness, as it does in man (6). To investigate these two questions, cats representing a range of susceptibility to motion were studied.

Materials and Methods

Cats were selected to represent three levels of susceptibility according to the degree of sickness observed when exposed to vertical linear acceleration on three previous biweekly selection trials. Five cats were selected to represent each susceptibility level. Cats that vomited during each selection trial were classified as susceptible (SUSC). Cats that failed to vomit during any selection trial were considered resistant (RESIS). A third group was classified as inconsistent (INCON) responders. These cats vomited during fewer than three of the selection tests, but did develop some symptoms of motion sickness (more than 3 points on the symptom rating scale for cats (10)) when vomiting did not occur. Animals were housed in the Ames Research Center Animal Care Facility and maintained on a 8.5:15.5 hour light:dark cycle with the lights coming on at 8:00 AM.

An indwelling catheter was implanted in an external jugular vein of each cat under Ketelar and Nembutal anesthesia. Catheters were implanted in the right vein when possible, although in some animals the left external jugular was used. Catheters were inspected daily and flushed periodically with a heparin-saline (50 U heparin/ml saline) solution. After flushing, the catheters were filled with heparin solution (1000 U/ml).

On days that blood was withdrawn, the animals were fasted for 5 h prior to the experiment. Acclimation to the test environment was accomplished by leaving each animal alone and undisturbed in the experimental room for 25 min prior to each experimental session. Test sessions began at 2:00 PM.

During testing, the animals were free to move inside a clear, ventilated plastic test cage (0.51 m long x 0.25 m wide x 0.33 m high). This test cage was attached to a platform suspended from an overhead support by three springs. Vertical sinusoidal motion was produced by moving the platform manually through a vertical plane of 0.6 m at a rate of 0.6 Hz for 20 min.

Just prior to each test, 0.5 ml of blood was withdrawn from the catheter to remove hemolized blood and heparin which would interfere with the radioimmunoassay. Samples were obtained by stopping the motion, opening the top of the test cage, withdrawing and discarding the contents of the catheter, and then withdrawing the blood to be assayed. For test samples, 1.5–2.0 ml of blood were withdrawn, expelled immediately into a 5 ml Vacutainer containing EDTA, mixed gently, and placed on ice until the end of the test session. The animal remained in the test cage during sampling. After completing each withdrawal the catheter was flushed with 1 ml of heparinized saline and motion was resumed. The time required to collect each sample was 50 s or less. A uniform 20-min exposure to motion was assured by stopping the motion timer while the vertical linear motion was stopped for blood withdrawal. Blood samples were withdrawn according to a predetermined schedule as shown in Table I. This procedure for collecting samples was also used in control (no motion) sessions in which the same animals were subjected to the same regimen but without the vertical linear acceleration. Motion and control sessions occurred in different pseudorandom orders separated by 14 d.

Following test sessions, samples were centrifuged at 4°C and 2000 rpm for 30 min. Plasma was removed and stored at −70°C until analyzed. Plasma AVP was radioimmunoassayed as described by Keil and Severs (5). Cortisol was determined using a radioimmunoassay kit (New England Nuclear).

Results

The average resting level for the hormones was determined for each animal by combining data from the four baseline samples (two samples from the motion and two from the control sessions). There is no distinction between motion and control sessions for these samples because animals were treated similarly prior to the onset of motion. The Kruskal-Wallis one-way ANOVA by ranks was used to test whether the resting level of hormones differed in the animals from the three susceptibility groups. Analysis of the data shows that there was no difference in the resting level of either AVP (median levels for the SUSC, INCON, and RESIS groups were 3.6, 4.0, and 3.0 $pg \cdot ml^{-1}$ plasma respectively, $H = 0.15$, $p > 0.10$) or cortisol (median levels for the SUSC, INCON, and RESIS groups were 2.2, 3.0, and 3.4 $\mu g \cdot dl^{-1}$ plasma, $H = 2.66$, $p > 0.10$) in animals from these groups. This result indicates that neither hormone is related to (i.e., predictive of) susceptibility of the cats to motion induced by vertical linear acceleration.

The levels of both AVP and cortisol in animals from all three susceptibility groups tended to increase during exposure to motion. The Kruskal-Wallis one-way ANOVA was applied to data from each of the four samples withdrawn during motion to determine whether levels of AVP and/or cortisol were related to levels of susceptibility. There were

TABLE I. SCHEDULE FOR WITHDRAWING BLOOD SAMPLES.

Sample Number	Type of Sample	Temporal Relationship to the Emetic Reflex
1 & 2	Baseline	10 & 1 min preceding start of motion
3, 4, 5, 6	Experimental	1, 5, 10, & 20 min after start of motion
7	Recovery	60 min after termination of motion

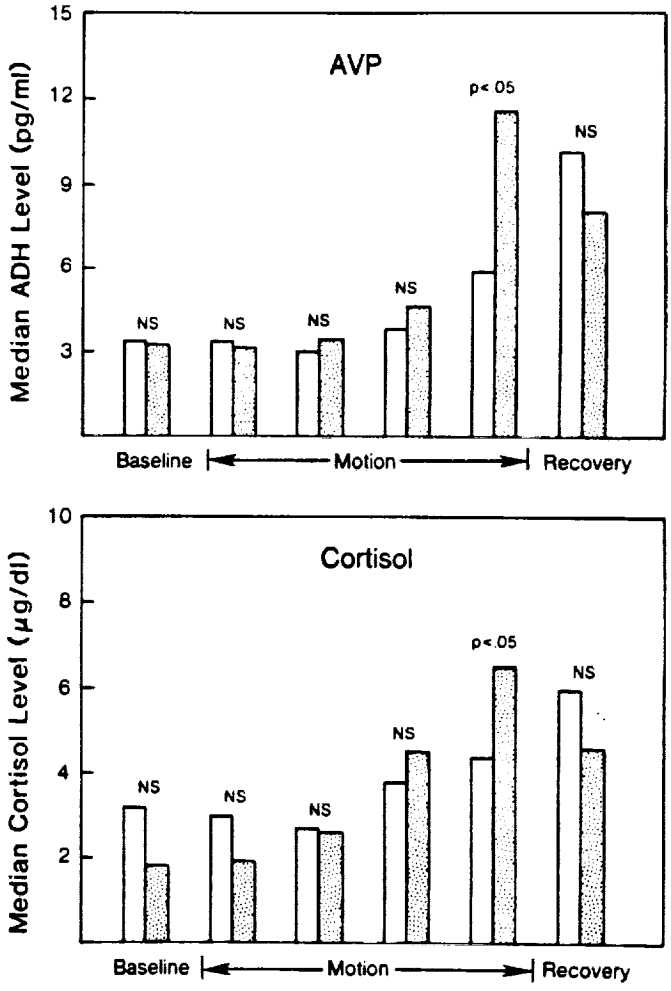

Fig. 1. The median values of AVP and cortisol for each sample in the control session (open bars) and motion session (stipled bars). The baseline value is the median of the average values for the two samples withdrawn before motion began. All other medians are based upon single samples.

no reliable differences in AVP (all $Hs < 3.20$, $p > 0.10$) or cortisol (all $Hs < 0.51$, $p > 0.10$) in any of these samples. Thus, changes in AVP and cortisol induced by motion appear to be equivalent for animals representing the three levels of susceptibility.

Because there were no differences in the levels of either hormone for the three groups on any sample during motion, the data from the three groups were combined to assess changes in the hormones produced by motion. The levels of AVP and cortisol based upon combined scores ($n = 15$) during motion and control sessions are shown in Fig. 1. In this figure, and in the following analyses, the average of the two samples withdrawn preceding the start of motion (or the equivalent samples in the control session) was taken as the baseline value (i.e., resting level) of the hormones. The Mann-Whitney U test was used to test whether levels in the motion session differed from levels in the control session. For both hormones, the levels during motion significantly exceeded control levels only in the sample collected 20 min after the start of vertical linear acceleration.

The Wilcoxon test was used to assess the apparent increase in the hormones in the recovery sample. Both AVP and cortisol were higher in the recovery than in the baseline sample (all $Ts < 16$, $p < 0.01$). Interpretation of this elevation of both AVP and cortisol is difficult for no measurements of plasma electrolytes or osmolality were made. In this experiment the cats were deprived of water for up to 6.5 h preceding the recovery sample. This is a relatively brief deprivation for osmotic-stimulation of AVP release, and it is not clear that this is a sufficient duration of deprivation to produce elevated AVP in the cat. Dehydration produced by restriction of water for 48 h does increase plasma osmolality and elevate AVP in the cat (8), but data for briefer periods of restriction were not provided. It should be noted, however, that the mechanism for release of AVP by osmotic influence is uncommonly sensitive in cats (8), and that up to 12 ml of blood were withdrawn in samples prior to the recovery sample in this experiment. Thus, the combination of blood loss and the brief period of water deprivation may have been responsible for the elevated hormone levels in the recovery sample.

EXPERIMENT II: SAMPLING PLASMA AVP AND CORTISOL AT THE TIME OF EMESIS

In the preceding experiment both AVP and cortisol were higher after 20 min of motion than after the same period in the control session. However, a clear understanding of the relationship between the two hormones and the emetic reflex is not provided by these data because blood samples were withdrawn according to a predetermined time schedule rather than in coordination with vomiting. Thus, the maximum rise in AVP may have been underestimated because the hormone was metabolized during the interval between vomiting and withdrawal of samples. This interval varied from one animal to another, further complicating interpretation of the data.

In this experiment, withdrawal of blood samples was keyed to the emetic response to allow a more accurate assessment of the relationship between emesis and plasma AVP levels. Animals were stimulated by motion to the point of vomiting. A blood sample was then withdrawn within 60 s. Motion was stopped when vomiting occurred so that recovery rates of cortisol and AVP could be determined in the absence of additional stimulation.

Materials And Methods

Four cats were prepared with indwelling catheters as in Experiment I. The fasting and acclimation procedures for

TABLE II. SCHEDULE FOR WITHDRAWING BLOOD SAMPLES KEYED TO THE EMETIC REFLEX.

Sample number	Type of Sample	Temporal Relationship to the Emetic Reflex
1	Baseline	10 min preceding start of motion
2	Experimental	Coincident with vomiting
3 & 4	Experimental	3 & 6 min after vomiting
5, 6, & 7	Recovery	25, 28, & 31 min after vomiting

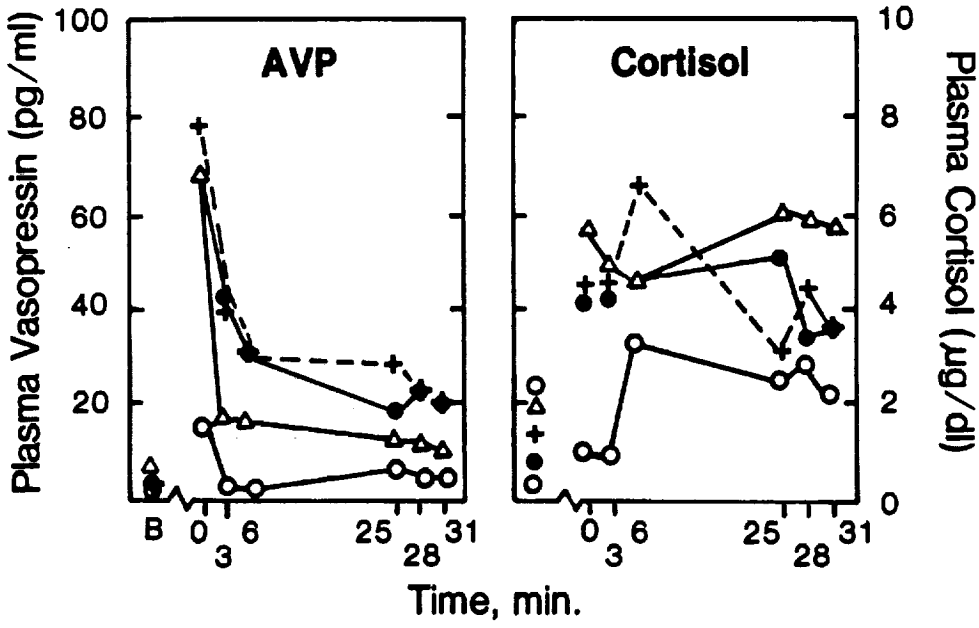

Fig. 2. Levels of AVP and cortisol for the four cats tested in Experiment II. Each curve reflects the data from the seven samples from one animal.

test days were the same as those described for the first experiment. In this experiment the test cage was attached to a motor-driven platform which produced sinusoidal vertical motion of 0.6 Hz and 0.5 m amplitude. Seven blood samples were withdrawn according to the sampling schedule shown in Table II.

Results

The levels of cortisol and AVP are shown for each animal in Fig. 2. Changes in plasma AVP are clarified by sampling at the time of vomiting. The AVP level is sharply elevated at the time of emesis (6 to 15 times the resting level). For each animal, the maximum AVP level occurred at the time of vomiting. Following vomiting the plasma AVP decreased to less than one-half of the maximum, and continued to decline gradually during the 31-min recovery period.

The level of cortisol also was elevated at the time of emesis (0.4 to 4.7 times the resting level), but unlike AVP, cortisol remained elevated throughout the session. Changes in cortisol appear to be of lesser magnitude relative to the resting value than those of AVP. In addition, changes in cortisol are smaller during emesis and return toward the resting value more slowly than do changes seen in AVP.

EXPERIMENT III: LEVEL OF AVP IN CSF

The data from these first two studies confirm that AVP rises in association with the emetic response in cats as it does in man when sickness is induced by exposure to motion (3,11) or by apomorphine administration (9). It is possible that plasma AVP may have a fluid conservation function, or that it may serve as a humoral link in the sequence of events leading to nausea and emesis. Such a humoral link has been proposed (1). To confirm such a mechanism it would be important to demonstrate that AVP concentrations in the brain are correlated with the emetic events. This experiment addressed the issue by assessing the levels of AVP in the CSF of cats.

Materials and Methods

Female cats were selected on the basis of their motion sickness susceptibility as determined by their responses to motion produced by a device similar to a miniature carnival ferris wheel (2). This device produces a gentle stimulus that does not appear to be stressful to the cat when operated at 0.28 Hz and 0.89 m amplitude for 30 min. The animals were exposed to this motion while housed individually in clear, ventilated plastic test enclosures (0.51 m long x 0.25 m wide x 0.33 m high).

Six cats were successfully implanted with chronic stainless steel cannulae directed stereotaxically to the rostral portion of the fourth ventricle just at its juncture with the cerebral aqueduct. Surgery was performed under intravenous pentobarbital anesthesia, and recoveries were uneventful. The cannulae were sharpened 18-gauge hypodermic needles cut to exact length. Hubs of the needles were sealed with obturators at all times except for the few seconds required to withdraw CSF through 22-gauge inner cannulae, which were cut to approximately the same length as the permanent cannulae.

Testing occurred between 9:15 a.m. and noon. The interval between surgery and times of sampling was from 7 to 30 d. Five samples of CSF were withdrawn during a motion test and another five samples for control data were taken the following day with the same time parameters but without motion. On both days, samples of 50 to 100 μl were withdrawn according to the schedule in Table III. Samples were quickly aliquoted into chilled vials which were, in turn, frozen on dry ice before storing at −80°C. Subsequently, all samples were shipped in dry ice to NASA/Ames by overnight mail for AVP radioimmunoassay.

TABLE III. SCHEDULE FOR WITHDRAWING SAMPLES OF CSF.

Sample Number	Type of Sample	Temporal Relationship to the Emetic Reflex
1 & 2	Baseline	20 & 10 min preceding motion
3	Experimental	Coincident with vomiting, or after 30 min of stimulation for resistant animals
4 & 5	Recovery	20 & 40 min after sample 3

Results

These data could not be analyzed with nonparametric statistics because of the small size of the samples. Thus, a 2 (retching/vomiting vs. not retching) x 2 (Motion vs. Control) x 5 (samples) mixed ANOVA with repeated measures on the last two factors was used to analyze the data. The relationship between resting levels of AVP in CSF and susceptibility to motion sickness was assessed by examining differences between cats that became sick vs. those that did not retch or vomit. The average level of AVP was higher in cats that did not retch/vomit (66.6 $pg \cdot ml^{-1}$, S.D. = 9.3) than in cats that did become sick (47.6 $pg \cdot ml^{-1}$ S.D. 3.4 [$F(1,4) = 11.13$, $p < 0.05$]). The relationship between motion sickness and central AVP was assessed by differences related to Motion and Control sessions, and differences among samples throughout the test sessions. Levels of AVP in Control and Motion sessions were not different ($F < 1$), and the level of AVP did not vary reliably among the five samples of the sessions [$F(4,16) = 2.75$, $p > 0.05$]. There was no striking elevation in AVP in Sample 3 when animals retched/vomited (mean = 40.8 $pg \cdot ml^{-1}$, S.D. = 11.8) in comparison with the same sample when the animals did not retch/vomit (mean = 48.0 $pg \cdot ml^{-1}$, S.D. = 10.8). Thus, there is no evidence in these data that AVP is elevated in CSF during (or immediately after) retching/vomiting. None of the interactions between variables produced effects which were statistically reliable ($p > 0.05$ for all tests).

GENERAL DISCUSSION

These experiments indicate that a dramatic elevation in plasma AVP is associated with the emetic reflex in cats. High levels of plasma AVP have been measured during nausea as well as at the time of vomiting in man (4,9). An association between AVP and nausea cannot be determined conclusively in animals because nausea is identified by self-report. However, when motion was continued following vomiting in Experiment I a high level of AVP was often maintained. This result is in contrast to the rapid decay of AVP seen when motion was terminated at the time of vomiting as shown in Experiment II. The continued high

level of AVP might reflect a prolonged state of sickness and/or nausea. If this interpretation is correct, the fact that AVP levels in susceptible and resistant cats exposed to motion in Experiment I were similar might suggest that although the resistant cats did not vomit, they did experience the physiological state of nausea.

The level of cortisol in plasma does not show a direct relationship to vomiting and perhaps reflects nonspecific stress induced by the experimental conditions rather than an effect of motion sickness. This finding is generally in agreement with the results of Eversmann *et al.* (3) who concluded that AVP is more closely related to motion sickness responses than is cortisol.

The relationship between susceptibility to motion sickness and resting levels of systemic AVP appears to be different in the cat than in man. Kohl *et al.* (6) reported that resting AVP levels may be lower in susceptible individuals than in those who are resistant. However, in this study with cats, measurement of the hormone in animals that differed greatly in their susceptibility to the motion failed to demonstrate a clear relationship between plasma AVP and susceptibility. Resting levels of plasma AVP were not different for susceptible and resistant animals. In addition, changes in the level of plasma AVP during stimulation were not reliably different in susceptible and resistant animals. However, resting AVP levels in CSF were lower in cats that retched/vomited during stimulation than in those that did not. Resting levels of CSF-AVP may be related in some, as yet unknown way to sickness induced by motion, or to central mechanisms related to the adaptability of individuals to abnormal motion conditions.

These experiments suggest that changes in plasma AVP are not accompanied by changes in the CSF-AVP levels. However, this interpretation must be made with caution because AVP levels in CSF and plasma were not determined simultaneously. A systematic evaluation of this point should be based upon time-dependent, simultaneous sampling of the hormones in both CSF and plasma. Since no changes were seen in CSF-AVP at or close to the time of vomiting, this experiment fails to provide evidence that vasopressin plays a role in the central stimulation of vomiting. However, this conclusion must be tempered because the number of tests made was small, and a relationship between CSF-AVP and emesis could be obscured if the timing and/or site of sampling of CSF were inappropriate.

It seems reasonable that vasopressin may serve a fluid conservation function during motion sickness. However, these data provide little or no support for the concept that AVP plays a causal role in vomiting induced by motion. The mechanism for the release of AVP during motion-induced vomiting remains unidentified and the very high levels of systemic AVP seen immediately after vomiting may reflect an association between the hormone and some unidentified factor causing motion sickness and a simultaneous release of vasopressin. A potential, but untested mechanism for this release could be stimulation of the thoracic baroreceptor system. Large fluctuations occur in thoracic blood pressure during the vomiting reflex (7), but a causal relationship between these changes and the release of vasopressin has not been examined. It should be noted that release of vasopressin by stimulation of the baroreceptors would not account for high levels of AVP during nausea in the absence of retching and vomiting (9). Thus, while the baroreceptor system may contribute to the release of AVP, it seems unlikely that this could be the sole cause of the phenomenon.

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Experiments I and II were conducted at Ames Research Center and conformed to the Center's requirements for the care and use of animals. Experiment III was conducted at Wright State University and followed the guidelines of the University for animal care and use. The requirements and guidelines of both institutions follow those developed by the National Research Council (1985).

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Effect of copper sulphate on the rate of afferent discharge in the gastric branch of the vagus nerve in the rat

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The afferent nerve activity was recorded from a nerve filament isolated from the peripheral cut end of the gastric branch of the vagus nerve. The gastric perfusion of 4 ml of two different concentrations (0.04% and 0.08%) of $CuSO_4$ solution provoked an increase in afferent activity. The stimulating effect of the 0.08% solution was stronger than that of the 0.04% solution, and lasted for a longer period of time. The observations suggest a possible mechanism by which $CuSO_4$ elicits emesis.

It has been reported by Wang and Borrison [5] that the emetic action of copper sulphate ($CuSO_4$) is two-fold, involving a central as well as a peripheral effect. Their report indicated that the interruption of the vagi had a more profound effect on the threshold and latency of vomiting than did sympathectomy, which caused no discernible changes in these parameters. They stressed that the vagal afferents play an important role in the mediation of the peripheral effects of $CuSO_4$. The present experiments were designed to follow up on these observations by investigating the effect of $CuSO_4$ on the rate of afferent discharge in the gastric branch of the vagus nerve.

Male Wistar rats weighing 300–400 g were used. Food, but not water, was removed 5 h before the experiment. Rats were anesthetized with 700 mg/kg of urethane and 50 mg/kg of chloralose, given i.p. A tracheal cannula was inserted.

The stomach could be perfused with $CuSO_4$ or physiological saline through a catheter which was placed in the esophagus and directed toward the cardiac portion of the stomach. Another catether was placed in the pyloric portion of the stomach through the duodenum as an outlet for the perfusate. Before starting the experimen-

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tal perfusions, the stomach was washed out with isotonic saline. Copper sulphate solutions of 0.04% and 0.08% and isotonic saline were used for the experimental perfusions. For each perfusion 4 ml of solution at 38°C were injected by syringe into the stomach over a 1-min period. The solution was kept in the stomach for 20 min, after which time the stomach was flushed for 1 min with isotonic saline.

The afferent nerve activity was recorded from a nerve filament isolated from the peripheral cut end of the ventral or dorsal branch of the vagus nerve. The nerve filament was placed on a pair of silver wire electrodes and immersed in a mixture of liquid paraffin and vaseline. Nerve activity was amplified by means of a condenser-coupled differential amplifier, and stored on magnetic tape. Analysis of nerve activity was performed after conversion of raw data to standard pulses by a window discriminator that distinguished the discharge of afferent fibers from background noise. To monitor the time course of changes in neural activity the rate of neural discharge was determined by a ratemeter with a reset time of 1 or 5 s. The output of this ratemeter was displayed on a pen recorder. Normal animal body temperature was maintained by means of a heating pad. The ECG was monitored throughout the experiment.

The effect of $CuSO_4$ on the afferent activity of the gastric branches of the vagus nerve was determined by comparing the mean number of spikes per second obtained over the 20 s (i.e. mean value of 20 successive measured samples) just before perfusion of $CuSO_4$ (baseline, firing rate), with those obtained 20 min after the onset of perfusion, and 30 min after flushing out the perfusate with isotonic saline. Statistical significance of differences in discharge rate was determined by Student's *t*-test.

The perfusion of 4 ml of two different concentrations (0.04% and 0.08%) of $CuSO_4$ solution provoked an increase in afferent activity of the gastric branch of the vagus nerve. After the onset of the perfusion with $CuSO_4$ the activity increased gradually and the increase lasted until after the flushing of the gastric canal with isotonic saline. The stimulating effect of the 0.08% solution of $CuSO_4$ was stronger than that of the 0.04% solution, and lasted for a longer period of time as shown in the upper trace of Fig. 1. With the 0.08% solution the increase in vagal activity lasted in general for more than 1 h, even though the stomach was flushed after 20 min of exposure to the $CuSO_4$. The peak of activity provoked by the $CuSO_4$ was reached after perfusate had been flushed out of the stomach (Fig. 1, upper and lower trace).

The effects seen on neural activity were not caused by mechanical effect of the infusion of solution into the stomach, since the perfusion of 4 ml of saline resulted in no noticeable increase in discharge rate beyond the transient increase that was observed at the onset of perfusion of both the $CuSO_4$ solutions and the saline (Fig. 1, lower trace).

Fig. 2 shows the mean discharge rate in spikes/s of 5 different preparations just before (control), 20 min after onset of 0.08% $CuSO_4$ perfusion, and 30 min after rinsing with saline. Those discharge rates are 6.4 ± 0.3 (S.E.M.), 13.4 ± 1.8 and 18.8 ± 2.3, respectively. The difference between firing rates obtained during the control period and the period 20 min after onset of perfusion, as well as between the control period and the period 30 min after rinsing were statistically significant (Fig. 2).

The experimental results indicate that gastric perfusion of 0.08% $CuSO_4$ solution

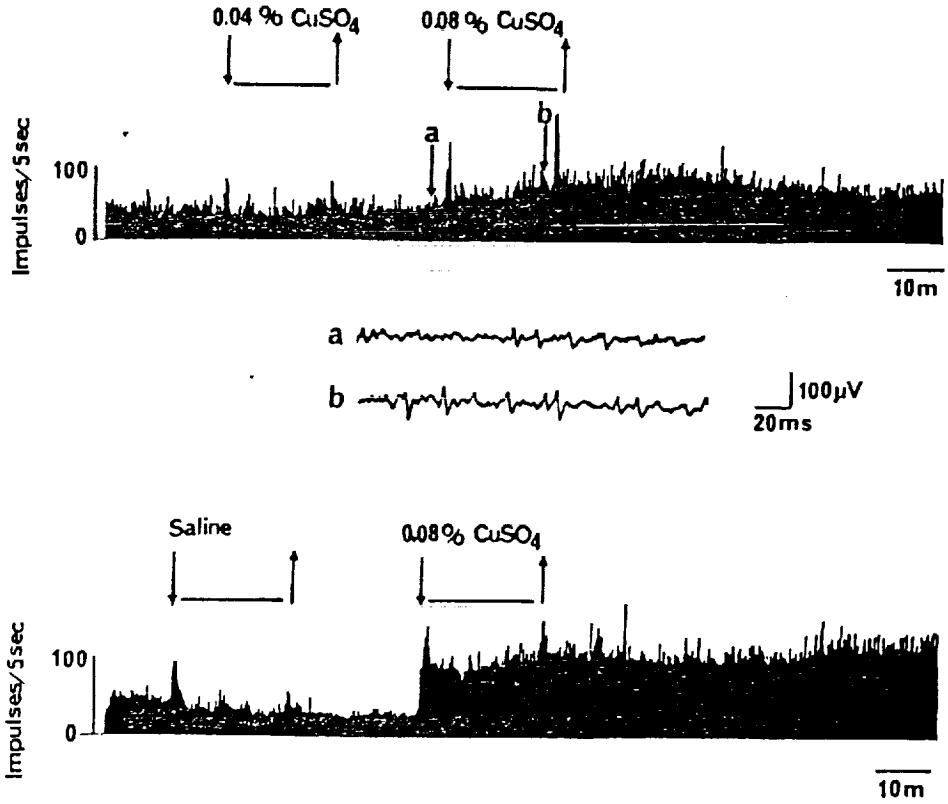

Fig. 1. Effect of gastric perfusion by 0.04% and 0.08% $CuSO_4$ solution and physiological saline on the afferent discharge rate of a vagal gastric nerve filament. Downward arrows show time of onset of perfusion. Upward arrows show the end of rinsing with saline. Horizontal bars indicate the duration of perfusion with $CuSO_4$ solution and physiological saline. a: sample of nerve activity taken at time indicated by arrow 'a', before perfusion with 0.08% $CuSO_4$. b: sample of nerve activity obtained at time indicated by arrow 'b', during perfusion with 0.08% of $CuSO_4$.

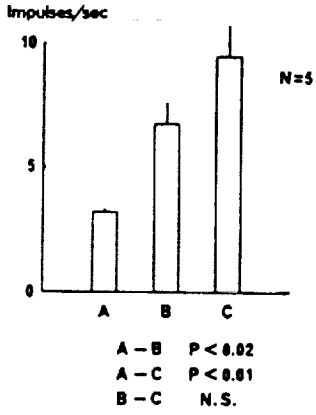

Fig. 2. Mean discharge rate of the gastric vagal afferents before (A), 20 min after onset (B) and 30 min after rinsing (C) of perfusion by 0.08% $CuSO_4$ solution.

increases afferent activity of the gastric branch of the vagus nerve. This finding suggests a possible physiological mechanism by which $CuSO_4$ elicits emesis. The failure to induce emesis using $CuSO_4$ after vagotomy (Oenchowski, quoted by Hatcher [1]) could thus be explained. The gradual increase in afferent discharge rate during $CuSO_4$ perfusion also suggests a physiological mechanism to explain the latency to emesis (9–15 min) following oral administration of $CuSO_4$.

It was established by Wang and Borrison [5] that the effective emetic concentration of $CuSO_4$ for oral administration was 0.08% in the dog and cat. The observations in this paper on the effect of $CuSO_4$ seem to be more consistent at the 0.08% than 0.04% concentration and might be expected to elicit vomiting more reliably at this concentration than at lower concentrations in the dog and cat.

The specific receptors mediating the gastric vagal afferent response to $CuSO_4$ have not yet been identified, although several candidates exist. Mei [4] has demonstrated the existence of vagal chemoreceptors in the intestinal wall, while Iggo [2] has suggested that gastric pH receptors exist. Mei [3] has also reported the existence of receptors in the mucous membrane of the gastrointestinal wall. While any of these receptors might be stimulated by $CuSO_4$ solutions, the exact source of the stimulating effect of $CuSO_4$ on gastric vagal afferents is not known. The existence of a specific receptor for substances that act as emetics, such as $CuSO_4$ and mustard, cannot be ruled out.

Wang and Borrison [5] reported that vagotomy increases the latency for emesis induced by orally administered $CuSO_4$. Their report mentioned that complete blockage of the emetic response to intragastric $CuSO_4$ required vagotomy combined with sympathectomy. They further suggested that the gastric splanchnic afferent pathway may play a role in the emetic response. Further electrophysiological observations must be made to determine whether a splanchnic afferent pathway is involved in emesis induced by $CuSO_4$ and/or other gastric irritants and to identify the receptors responsible for the effects of $CuSO_4$.

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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.

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 esophagogastric 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 opthalmic 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 1. 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 1
Descriptive Statistics for the Two Measures Used to Assess the Completeness of Vagotomy

Measure	Statistic	Experimental group		
		Vagotomy	Ligation	Intact
Weight Change (percentage)	Mean	−23.6	−16.8	−3.7
	Range	−27 to −18	−27 to −6	−12 to +2
Stomach Contents (grams)	Mean	4.6	0.2	0.4
	Range	2.6 to 7.7	0.04 to 0.43	0.09 to 0.90

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

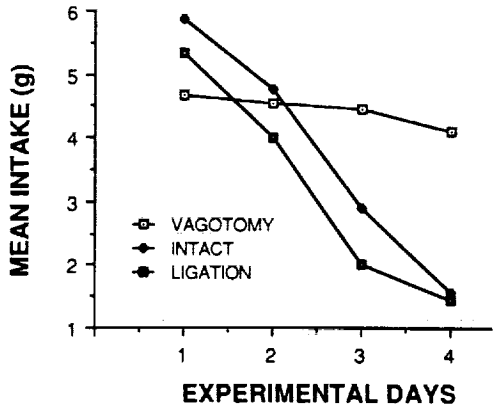

FIG. 1. Mean intake of vinegar-flavored water by the three groups in the four drinking sessions. On each experimental day flavored water was drunk immediately before 30 min of rotation. Thus, intake on Day 1 is a baseline preceding conditioning, and the intake on each of the remaining experimental days reflects conditioning effects of rotation from the preceding experimental day.

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, $F(2, 24) < 1$. A 3 (groups) × 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 [for the comparison of all succeeding days with Day 1, 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 produced 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. Coil 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 (Niijima, 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.

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Basic and Applied Aspects of Vestibular Function
J.C. Hwang, N.G. Daunton and V.J. Wilson (Eds.)

IMMUNOCYTOCHEMICAL LOCALIZATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) AND SUBSTANCE P IN NEURAL AREAS MEDIATING MOTION-INDUCED EMESIS. EFFECTS OF VAGAL STIMULATION ON GAD IMMUNOREACTIVITY

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Abstract

Immunocytochemical methods were employed to localize the neurotransmitter amino acid γ-aminobutyric acid (GABA) by means of its biosynthetic enzyme glutamic acid decarboxylase (GAD) and the neuropeptide substance P in the area postrema (AP), area subpostrema (ASP), nucleus of the tractus solitarius (NTS) and gelatinous nucleus (GEL). In addition, electrical stimulation was applied to the right vagus nerve at the cervical level to assess the effects on GAD-immunoreactivity (GAD-IR).

GABA: GAD-IR terminals and fibers were observed in the AP, ASP, NTS and GEL. They showed pronounced density at the level of the ASP and gradual decrease towards the solitary complex. Nerve cells were not labelled in our preparations. Ultrastructural studies showed symmetric or asymmetric synaptic contacts between labelled terminals and non-immunoreactive dendrites, axons or neurons. Some of the labelled terminals contained both clear- and dense-core vesicles. Our preliminary findings, after electrical stimulation of the vagus nerve, revealed a bilateral decrease of GAD-IR that was particularly evident at the level of the ASP.

Substance P: SP-immunoreactive (SP-IR) terminals and fibers showed varying densities in the AP, ASP, NTS and GEL. In our preparations, the lateral subdivision of the NTS showed the greatest accumulation. The ASP showed medium density of immunoreactive varicosities and terminals and the AP and GEL

Keywords: GABA, substance P, vagus nerve, area postrema, nucleus tractus solitarius, immunocytochemistry, electrical stimulation

displayed scattered varicose axon terminals. The electron microscopy revealed that all immunoreactive terminals contained clear-core and dense-core vesicles which made symmetric or asymmetric synaptic contact with unlabelled dendrites.

It is suggested that the GABAergic terminals might correspond to vagal afferent projections and that GAD/GABA and substance P might be co-localized in the same terminal allowing the possibility of a regulated release of the transmitters in relation to demands.

Introduction

The present report is part of a study designed to investigate the interaction between neuropeptides and conventional neurotransmitters under conditions producing motion sickness and in the process of sensory-motor adaptation.

A vast amount of literature has dealt with the cytoarchitectural organization and ultrastructural analysis of the area postrema (AP), area subpostrema (ASP), nucleus of the tractus solitarius (NTS) and gelatinous nucleus (GEL), all structures localized in the dorsal part of the medulla oblongata (e.g., Olszewski and Baxter, 1954; Taber, 1961; Gwyn and Wolstencroft, 1968; Klara and Brizzee, 1975, 1977; Chernicky *et al.*, 1980; D'Amelio *et al.*, 1986). Anatomical studies have provided details of their somatotopic organization in relation to visceral afferents and physiological findings have demonstrated their involvement in a variety of autonomic functions (e.g., von Euler *et al.*, 1973; Gwyn *et al.*, 1979; Gwyn and Leslie, 1979; Katz and Karten, 1979; Gale *et al.*, 1980; Hamilton and Gillis, 1980; Helke *et al.*, 1980; Kalia and Mesulam, 1980a, b; Panneton and Loewy, 1980; Ciriello *et al.*, 1981; Kalia, 1981; Kalia and Sullivan, 1982; Helke, 1982). Neurotransmitters such as GABA, catecholamines, neuropeptides and serotonin have been identified by immunocytochemical procedures (e.g., Armstrong *et al.*, 1981, 1982a, b; Maley and Elde, 1982; Maley *et al.*, 1983; Kalia *et al.*, 1984; Maley, 1985; Maley and Newton, 1985; Newton *et al.*, 1985; D'Amelio *et al.* 1987; Maley *et al.*, 1987; Newton and Maley, 1987; Nomura *et al.*, 1987) and in some cases, synaptic interactions between neurotransmitters have been established (Pickel *et al.*,1979, 1984; Kubota *et al.*, 1985). By combining autoradiography and immunocytochemistry, Sumal *et al.* (1983) reported synaptic interactions between vagal afferents and catecholaminergic neurons in the NTS of the rat. Glial fibrillary acidic protein and glutamine synthetase were identified in the glioependymal cells and astrocytes of the cat AP (D'Amelio *et al.*, 1985, 1987). The relevance of the AP as the emetic chemoreceptor trigger zone has been corroborated (Borison and Brizzee, 1951; Carpenter *et al.*, 1983; Borison *et al.*, 1984) and evidence of its participation in the emetic response to motion has also been reported (Wang and Chinn, 1952, 1954; Brizzee *et al.*, 1980; Crampton and Daunton, 1984).

In this report we will describe the light microscopic distribution and ultrastructural appearance of GAD- and SP-immunoreactivity, the preliminary observations on the effects of electrical stimulation of the vagus nerve on GAD-IR and discuss some of our views with respect to the relationship between neurotransmitter action and distribution pattern and degree of density of the immunoreactive structures.

Material and Methods

Animals

Adult cats were employed for this study. They were housed in air-conditioned rooms and given regular dry pellet cat food and water *ad libitum.*

Antisera

The well characterized GAD-antiserum (code #P3) was kindly provided by Dr. Jang-Yen Wu (Pennsylvania State University, Hershey Medical Center, Hershey, PA) (for review, see Wu *et al.*, 1982).

The monoclonal antiserum for substance P was obtained from Pel-Freeze Biologicals, code MAS 035b.

Immunocytochemical Procedures

The peroxidase-antiperoxidase method developed by Sternberger (1979) was employed to visualize the immunoreactivity of both GAD and SP. Dilution of antisera was 1:1000. The details of the general procedures concerning fixation of the brain and treatment of the sections have been previously published (D'Amelio *et al.*, 1987).

Electrical Stimulation of the Vagus Nerve

The animals were tranquilized with an intramuscular injection of 0.5 ml ketamine HCl, after which they were anesthetized with an intravenous injection of sodium pentobarbital (30-35 mg/kg). The right cervical vagus nerve was exposed between the branching points of the superior pharyngeal and the recurrent nerves. Bipolar electrodes were positioned on the nerve and biphasic square-wave pulses of 0.6 sec duration were applied at 1-10 ma and 60 Hz. The current was steadily increased from 1 ma to 10 ma to determine a threshold response from the nerve. The threshold response was hyperventilation as observed visually or recorded on a polygraph via a respirometer. The nerve was stimulated continuously for a total duration of one hour. During the last 5 minutes of stimulation the thoracic cavity was opened and the perfusion procedure via the heart was started (see D'Amelio *et al.*, 1987).

Results

GAD Immunocytochemistry

The details of GAD-immunoreactivity in the AP, ASP, NTS and GEL have been published elsewhere (D'Amelio *et al.*, 1987). Briefly, the light microscopic examination revealed variable degree of density of the GAD-IR terminals and fibers along the rostrocaudal axis. Their distribution is exemplified in Figures 1A, B and C.

It is obvious that the ASP is distinguished from the AP and NTS by its high concentration of GAD-IR pre-terminal fibers and boutons which are seen at all levels examined. No labelled neurons were observed in our preparations.

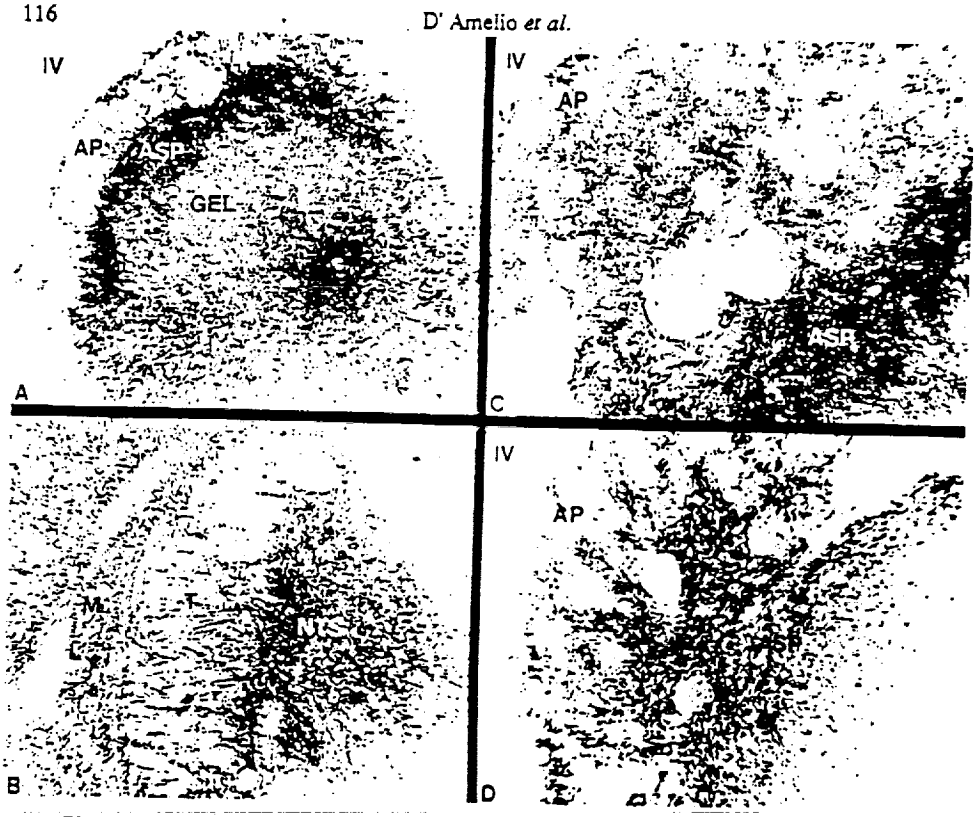

Fig. 1. *A*: Rostral segment at the level of the area postrema (AP). The area subpostrema (ASP) is distinguished by the high density of GAD-IR terminals. The decrease in density is evident towards the gelatinous nucleus (GEL). *B*: GAD-IR is present in the lateral sub-division of the nucleus of the tractus solitarius (NTS). The medial region of the nucleus (M) shows lighter immunoreactivity. *C*: Medial segment of the AP. Patches of GAD-IR terminals are visible throughout the AP. The high density of the ASP is also apparent at this level. *D*: Medial segment of the AP. In both the AP and ASP there is extreme depletion of immunoreactivity after electrical stimulation. Only scattered GAD-IR immunoreactive boutons are visible in the ASP. IV, fourth ventricle; T, solitary tract. Magnifications: *A*, x150; *B*, *C* and *D*, x250.

The ultrastructural study demonstrated that the GAD-IR boutons corresponded to immunoactive terminals with occasional staining of the pre-terminal segment. The immunoprecipitate outlined clear synaptic vesicles, mitochondria and the inner surface of the plasma membrane. Many of the profiles contained dense-core vesicles in variable number. Synaptic contacts, either symmetric or asymmetric were observed between labelled terminals and unlabelled dendrites, axons or neurons (Fig. 2).

The most ostensible finding of our preliminary observations after electrical stimulation of the vagus nerve was a noticeable bilateral decrease in density of the GAD-IR terminals of the ASP. In non-stimulated animals the density of these terminals was clearly higher in ASP than in the underlying structures. The decrease with stimulation seemed to involve all levels

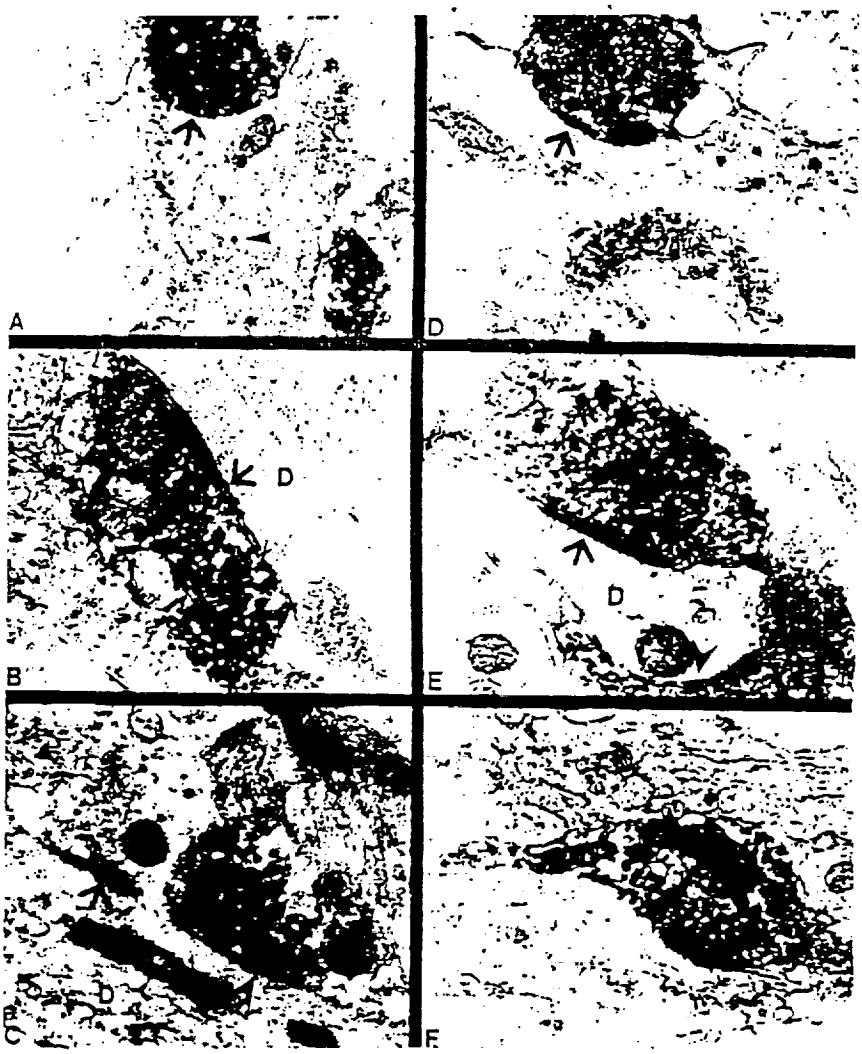

Fig. 2. *A*: Symmetric synaptic contact (arrow) between a GAD-IR axon profile and an unlabelled axon containing clear-core vesicles and scattered dense-core vesicles (arrowhead). *B*: GAD-IR profile forms a long symmetric contact with a dendrite (D). *C*: A GAD-IR axon terminal which contains clear-core vesicles and a few dense-core vesicles forms symmetric contact with a dendrite (arrowhead). In close apposition to the immunoreactive terminal is seen a non-immunoreactive axon profile forming an asymmetric contact with the same dendrite (arrow). *D*: Symmetric contact (arrow) between a GAD-IR axon profile containing both clear- and dense-core vesicles and a dendrite. *E*: Asymmetric (arrow) and symmetric (arrowhead) contacts between two GAD-IR terminals with the same dendrite (D). *F*: GAD-IR pre-terminal segment and bouton. Several dense-core vesicles are seen in addition to the clear-core ones. Magnifications: *A*, x16,000; *B*, x25,000; *C* and *D*, x20,500; *E* and *F*, x25,000.

of the ASP (Fig. 1D). The level of GAD-IR in the AP, NTS and GEL also showed a decrease in stimulated animals, with some inter-animal variation in the different regions. This finding has to be evaluated with further quantitative assessment.

Substance P Immunocytochemistry

The pattern of immunoreactivity of this neuropeptide within the AP, ASP and NTS is largely consistent, with some variations, with that found by other investigators (Maley and Elde, 1982; Newton *et al.*, 1984). In the ventromedial part of the ASP, SP-IR punctate structures and varicose axons appeared to be more abundant than in the dorsolateral region. In the AP, NTS and GEL, varying densities of immunoreactivity were found along the rostro-caudal axis, arranged into aggregates of ill-defined boundaries. The AP proper contained mainly varicose terminals, randomly distributed. In our preparations the NTS exhibited labelled terminals and varicosities in all topographical subdivisions with distinct pronounced density in the lateral subdivision. We did not find labelled neurons in any of the regions under study (Fig. 3).

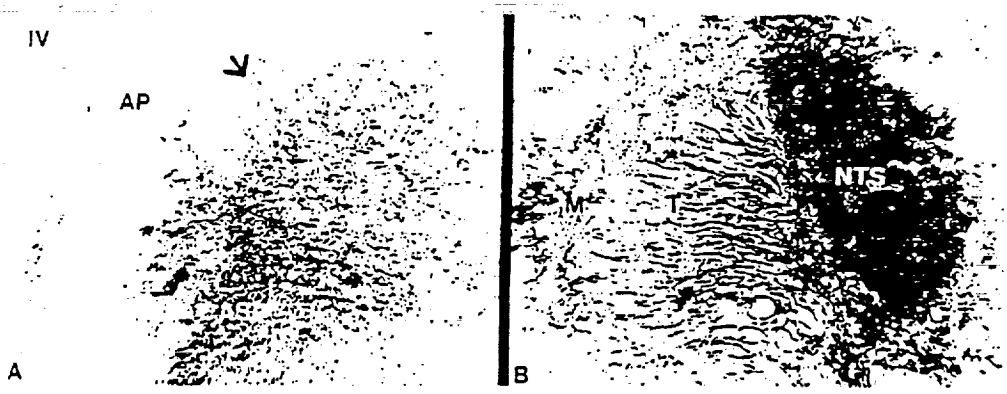

Fig. 3. *A*: SP-IR terminals are seen in the ASP, particularly towards the ventromedial region. A decrease in density is observed dorsolaterally. Scattered patches of immunoreactive terminals are seen in the AP proper (arrow). *B*: The pronounced density of SP-IR terminals and fibers is apparent in the lateral sub-division of the NTS and contrasts with the light immunoreactivity of the medial region (M). T: solitary tract. Magnifications: *A* and *B*, x250.

We concentrated our electron microscopic studies mainly on the AP and ASP. The most significant finding was that all the immunoreactive terminals contained dense-core vesicles in variable number (2-10) together with clear-core vesicles bound by immunoprecipitate. The majority of the dense-core vesicles showed immunoreactivity. The synaptic contacts were mainly between labelled axons and dendrites, either symmetric or asymmetric (Fig. 4).

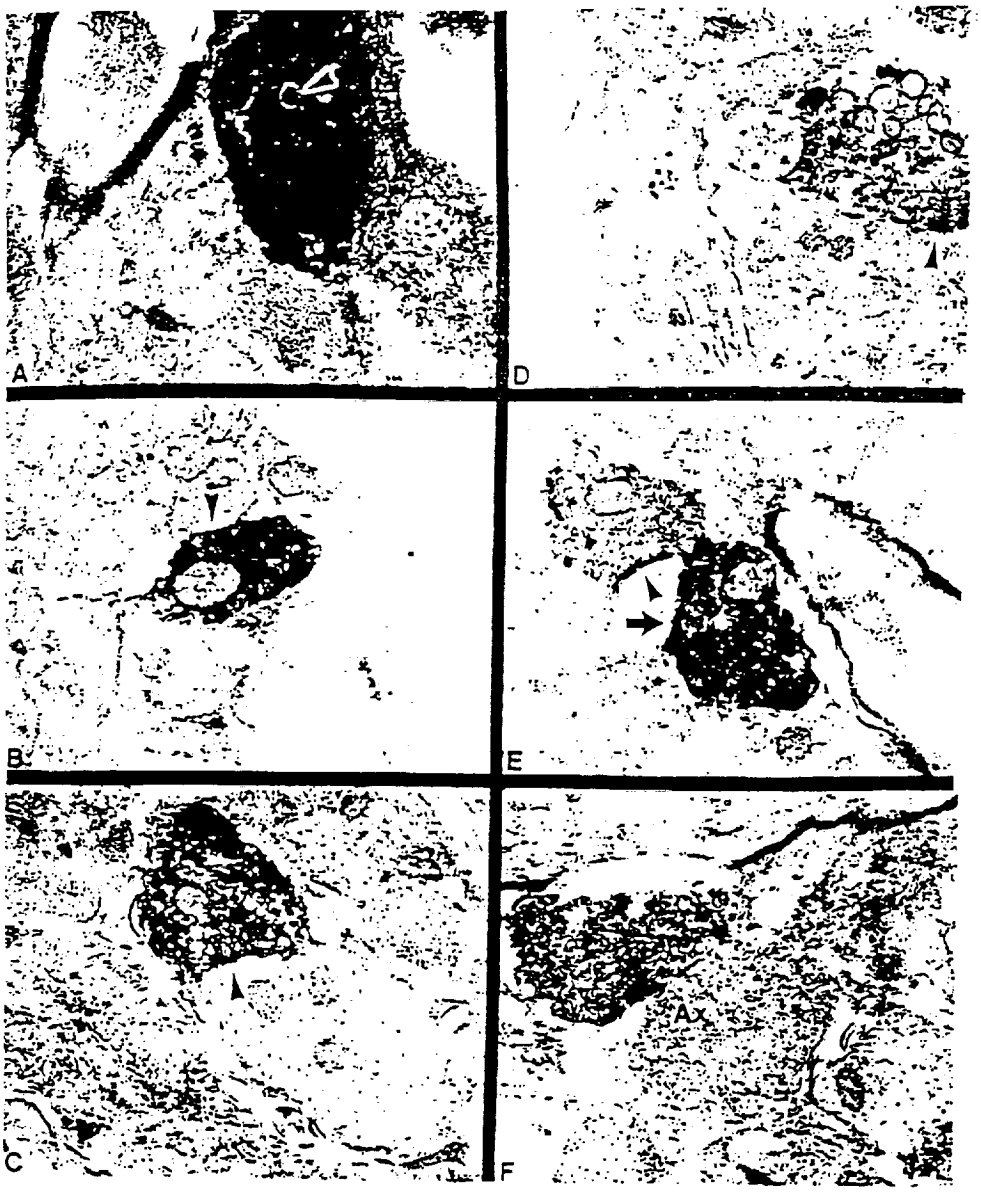

Fig. 4. *A*: SP-IR immunoreactive bouton in which the dense-core vesicles are markedly immunoreactive, except for one (arrowhead). *B*, *C* and *D*: Symmetric synaptic contacts beween SP-IR terminals and unlabelled dendrites (arrowheads). *E*: Two SP-IR terminals, one weakly labelled (arrowhead) and the other strongly immunoreactive (arrow), forming symmetric contacts with the same dendritic profile. *F*: Two axon terminals, one SP-IR and one unlabelled (Ax), in close apposition. Magnifications: *A*, x25,000; *B* and *C*, x20,500; *D*, x16,000; *E* and *F*, x20,500.

Discussion

It is our contention that extreme caution should be applied in assessing the distribution and 'mapping' of neurotransmitters in the central nervous system by means of immunocytochemical techniques. It is frequently neglected that the difference in immunocytochemical images in various areas of the brain obtained by different investigators is, in many instances, not the product of methodological procedures, source or sensitivity of antisera, etc., but of the dynamic character of intercellular signaling among neurons, which also frequently accounts for inter-animal variation. This signaling is the reflection of the actual 'motion' of neurotransmitters within a functional system in response to external (environmental) or internal (homeostatic changes) conditions which in turn might affect the rate of biosynthesis of the transmitter or its precursor and hence its release. In consequence, we prefer to consider the distribution of a particular neurotransmitter as 'provisional' and rely upon procedures such as tract-tracing methods, autoradiography, and physiological methods, combined with ultrastructural and light microscopic immunocytochemistry, to try to define communication lines among neural regions.

Following this line of argument for the region under study, we think that the distribution pattern of GABAergic terminals in the AP, NTS, ASP, and gelatinous nucleus, closely resembles that of vagal afferent projections found by means of horseradish peroxidase (HRP) injections of the proximal cut ends of the vagus nerves (Ciriello *et al.*, 1981), following HRP or [^{3}H]leucine injections of the nodose ganglion (Gwyn *et al.*, 1979) and with the use of degeneration methods after the removal of the nodose ganglion (Gwyn and Leslie, 1979). Furthermore, our preliminary findings of the depletion of GAD-IR in those areas after electrical stimulation of the vagus nerve seem to confirm that at least part of the GABAergic projections correspond to vagal afferents. The bilaterality of the GAD-immunoreactivity depletion seems also to coincide with the tract-tracing studies of Kalia and Mesulam (1980), who found bilateral sensory labelling of the AP and NTS after HRP injections of the right nodose ganglion. There also appears to be evidence that the depletion in GAD-IR is not due to a widespread effect of the vagal stimulation, since some areas of the histological sections, e.g., the ß nucleus of the inferior olive, show prominent GAD-IR in both non-stimulated and stimulated cats. As for the causes of GAD-IR depletion, it is an early stage in this research to attempt an explanation, since the analysis of sub-cellular and molecular mechanisms has not been initiated.

With respect to SP-IR in the AP, NTS and ASP, it is interesting to notice that although the density gradients differ from those of GAD-IR, they follow a similar pattern of localization, with the lateral sub-division of the NTS showing the greatest accumulation of SP-IR terminals and fibers. At this point, and in keeping with the opening remarks of our discussion, it is important to emphasize that it is not density, considered in a rigid context, of immunoreactive fibers and terminals that is expected to provide meaningful data to assess the functional significance of neurochemical phenomena in a given area of the nervous system. For example, in previous studies dealing with the immunoreactivity of substance P in the NTS of the cat (Maley and Elde, 1982) and Rhesus monkey (Maley *et al.*, 1987), it was reported that the respiratory subdivisions displayed a low level of immunoreactivity. These findings led the researchers to speculate that substance P does not play a major role in the mediation of respiratory functions. However, measurements of substance P by microdialysis

in the cat NTS (Lindefors *et al.*, 1986) and microinjections of substance P in the NTS of the rat (Carter and Lightman, 1985) have supplied significant evidence for the relevance of substance P in respiratory functions. In our opinion, the significance of the presence of a neurotransmitter within a particular structure will not be properly understood until its source and synaptic relations with other functional systems are clearly defined.

One feature that deserves to be stressed is the presence in all SP-IR terminals of dense-core vesicles, a characteristic already shown in the NTS and other regions of the nervous system (Maley, 1985; Pickel *et al.*, 1979). The possibility of peptide storage by those vesicles has been suggested by Pickel *et al.* (1979). Interestingly, many GAD-IR terminals also contain dense core vesicles in addition to the clear-core ones. This observation suggests that both messengers, GABA and substance P, may coexist within the same terminal, as has been previously reported for other areas of the nervous system. For example, 95-98% of SP-IR cortical neurons have been found to be also immunoreactive for GABA and GAD (Jones and Hendry, 1986). Additional observations account for the possibility of such co-existence. Immunocytochemical studies of substance P in other species have shown its presence within the neurons of the nodose ganglion, which is known to send sensory projections to the solitary complex and AP (Katz and Karten, 1980). Since, according to our observations and those of others, both GAD and substance P are consistently present in those regions, it is reasonable to assume that the sensory cells of the nodose ganglion might regulate the genetic expression of GABA, substance P and their biosynthetic enzymes. Our own preliminary studies after infra-nodose electrical stimulation of the vagus nerve provide further support to this hypothesis. Naturally, the possibility of the existence of separate neuronal populations in the nodose ganglion expressing either GAD/GABA or substance P, cannot be excluded.

The co-existence of both neuronal messengers in fibers and terminals of the region under study would once again demonstrate the extensive scope of the transmission process and the adaptive capacities of brain circuitry, with variable and regulated responses for the release of a neurotransmitter (or neuromodulator) according to the imposition of a given stimulus.

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Basic and Applied Aspects of Vestibular Function
I.C. Hwang, N.G. Daunton and V.J. Wilson (Eds.)

THE EFFECTS OF AREA POSTREMA LESIONS AND SELECTIVE VAGOTOMY ON MOTION-INDUCED CONDITIONED TASTE AVERSION

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Abstract

Conditioned taste aversion (CTA) is one of several behaviors which has been suggested as a putative measure of motion sickness in rats. A review is made of studies which have used surgical disruption of area postrema or the vagus nerve to investigate whether CTA and vomiting induced by motion may depend on common neural pathways or structures. When the chemoreceptive function of the area postrema (AP) is destroyed by complete ablation, rats develop CTA and cats and monkeys develop CTA and vomit. Thus the AP is not crucially involved in either CTA or vomiting induced by motion. However, after complete denervation of the stomach or after labyrinthectomy rats do not develop CTA when motion is used as the unconditioned stimulus. Studies of brainstem projections of the vagus nerve, the area postrema, the periaqueductal grey, and the vestibular system are used as the basis for speculation about regions which could mediate both motion-induced vomiting and behavioral food aversion.

Introduction

Animals commonly avoid the ingestion of foods treated with non-lethal doses of poison. The laboratory study of this phenomenon has led to the development of specialized procedures for investigating the role learning plays in this behavioral aversion to poisoned food. These procedures commonly are referred to as the 'conditioned taste aversion paradigm'. In typical applications of this paradigm a previously novel food is ingested just prior to poisoning. This 'pairing' of food with the effects of poisoning results in a strong, long-lasting avoidance of

Keywords: conditioned taste aversion, vomiting, area postrema, vagus nerve, reticular formation, vestibular system, periaqueductal grey, nucleus tractus solitarius

Neural Structures

Surgical lesions have been used in numerous experiments to investigate the neural structures crucial to CTA and vomiting. Usually, such studies have been used to investigate the effects of lesions on vomiting or on CTA independently, but not upon both responses simultaneously. Many of these studies have focused upon the AP and the vagus nerve. Thus, in independent studies it has been shown that the AP is critically involved in the emetic and in the conditioning efficacy of certain toxins. These effects commonly are attributed to a chemoreceptive function of the AP (Coil and Norgren, 1981; Carpenter *et al.*, 1983; Borison *et al.*, 1984). In addition, involvement of the vagus nerve has been shown in both vomiting (Borison, 1952) and CTA (Coil *et al.*, 1978; Rabin *et al.*, 1985) induced by intra-gastric copper sulfate.

Studies conducted as direct examinations of the role of the AP and the vagus nerve in motion-induced CTA and as simultaneous evaluations of the relationship been CTA and vomiting have occurred only recently. It has been known for some time that rotation could be used as a US to induce CTA in rats (Braun and McIntosh, 1973; Green and Rachlin, 1973). Because the AP had long been thought necessary for motion-induced vomiting in dog, cat, and monkey, and because the AP was known to be involved critically in the induction of CTA by certain toxins and radiation, the role of the AP in motion-induced CTA was investigated first. Ossenkopp (1983) reported that rats with the AP ablated formed a stronger CTA than unoperated rats when saccharin was paired with motion. He proposed that this enhanced CTA could have occurred because ablation of AP influenced the intake of the saccharin (a preferred fluid) which was used as a CS. A second study (Sutton *et al.*, in press) also demonstrated that motion can be used to induce CTA in rats with the AP ablated, but did not find enhanced CTA in ablated rats when a cider vinegar solution was used as the CS. In these two studies conditioning failed to occur when blood-borne toxins were used as the US (scopolamine methyle nitrate and lithium chloride, respectively), thereby indicating that the chemoreceptive function of the AP was eliminated by the ablations. Thus, in rats, the AP apparently is not a chemoreceptive site of action for a neurohumoral substance critical to motion-induced CTA.

Recent ablation studies have demonstrated clearly that the AP is not required for motion-induced vomiting in cats (Corcoran *et al.*, 1985; Borison and Borison, 1986) or squirrel monkeys (Elfar *et al.*, 1986; Wilpizeski *et al.*, 1986). Vomiting and CTA have been assessed in the same animals after ablation of the AP in three experiments. After ablation of the AP in cats, neither vomiting nor CTA was produced by a dose of xylazine which reliably produces vomiting in cats (Corcoran *et al.*, 1985). Vertical linear acceleration did produced vomiting on some trials, and CTA was produced when this motion was used as the US with these same AP-ablated cats. In squirrel monkeys with AP ablated, CTA was not produced by an intraperitoneal injection of LiCl, a chemical which requires an intact AP to produce CTA in rats (Ritter *et al.*, 1980; Sutton *et al.*, in press). However, these same monkeys vomited in some tests when exposed to vertical axis rotation, and CTA was produced by this motion stimulation (Elfar *et al.*, 1986). In the second study with squirrel monkeys, conditioned aversion was not investigated with chemical toxicosis, but rotation did produce CTA in monkeys with the AP ablated (Wilpizeski *et al.*, 1986). These studies provide additional support for an important chemoreceptive function of the AP in both emesis and CTA induced

by certain chemicals, and they simultaneously demonstrate that the emetic and taste aversion-producing properties of motion are not crucially dependent upon this chemoreceptive function of the AP.

The effect of disruption of the vagus nerve upon motion-induced CTA has been reported only in rats (Fox and McKenna, in press). In this experiment gastric denervation was accomplished by sectioning the anterior branch of the vagus distal to the hepatic branch, ligating and sectioning the posterior branch and the gastric artery proximal to the esophagogastric junction and then sectioning vagal branches in the region of the cardiac sphincter and along the greater curvature of the stomach. After this selective gastric vagotomy CTA was not produced when vertical axis rotation was the US. Animals in a control group subjected to ligation of the gastric artery and posterior vagus developed a CTA equal in magnitude to the aversion developed in unoperated animals. Becuase of this effect, it was proposed that either the anterior vagus, the sympathetic fibers, or both are crucial for motion to be an effective US for CTA in rats. Thus, while ablation of the AP has no apparent effect upon CTA produced by motion in rats, cats, or squirrel monkeys, the efficacy of rotation as a US for CTA in rats is disrupted after complete gastric denervation. The possibility that vagal pathways might be shared by CTA and vomiting could not be addressed directly in this experiment because rats are incapable of vomiting.

These investigations have shown that the AP plays no critical role in motion-induced CTA or vomiting. In some studies it was shown that motion produced both vomiting and CTA after the chemosensory function of the AP was eliminated by ablation. Thus, as has been asserted for vomiting (Borison, 1985), CTA induced by motion apparently does not depend upon a humoral factor acting on the AP. The question of whether CTA and vomiting depend upon common neural structures remains unanswered by these studies because both responses were unaffected by ablation of the AP.

Inferences regarding a role for gastric innervation can be only speculative at this time. Gastric denervation eliminates the efficacy of motion as a US for CTA in the rat, but the processes underlying this effect are unclear. Both afferent and efferent vagal functions were eliminated by gastric denervation, and secondary effects of this disruption on the CNS were not assessed. In addition, the magnitude of CTA produced by motion is reduced greatly when the labyrinth is destroyed in rats (Hartley, 1977). Thus, both labyrinthine and gastric systems contribute critically to the support of CTA induced by motion, and neither vestibular nor gastric inputs to the CNS alone is adequate for the production of CTA when motion in the US in the rat. Because CTA can be produced by motion only when both systems are intact, it seems that vagal and labyrinthine circuitry either must converge in some CNS region which is necessary for the support of motion-induced CTA, or alternatively, some form of modulation occurs between the two systems. Caloric stimulation of the labyrinth influences the rate of efferent activity in the vagus nerve of rats (Niijima *et al.*, 1988), and, in man, gastric emptying is delayed and duodenal motility is reduced by vestibular stimulation (Thompson *et al.*, 1982), further indicating interaction of the two systems.

A CNS locale where vagal and vestibular fibers may interact is unknown. Vagal afferents project to the subnucleus gelatinosus, the medial NTS, and the commissural NTS (Leslie *et al.*, 1982; Shapiro and Miselis, 1985). Dendrites of dorsal motor nucleus neurons have been reported to be co-distributed with these afferent projections and to penetrate the ependyma of the fourth ventricle and the ventral aspect of the AP. This co-distribution of afferent and

efferent components of the gastric vagus has been suggested as a possible locale for monosynaptic vagovagal interactions (Shapiro and Miselis, 1985). It has also been shown that cells in the medial half of the medullary parvicellular reticular formation (PCRF) project to the caudal solitary and vagal nuclei in the cat (Mehler, 1983). The PCRF is a site of origin of efferent fibers projecting to the vestibular sensory epithelium and it has been speculated that these efferents may contribute to a vomiting trigger zone circuit via the generation of a mismatch signal with vestibular afferent signals (Goldberg and Fernández, 1980; Mehler, 1983). The PCRF also receives projections from the periaqueductal grey, a necessary structure for the production of CTA when morphine is the US (Blair and Amit, 1981).

Conclusions

These studies demonstrate that neural fibers associated with the periaqueductal grey, the vestibular system, the AP, and the stomach, four structures which have been demonstrated to be important to CTA produced by various USs, are found in the NTS and PCRF. The NTS and PCRF are characterized by complicated interconnections locally, and with higher brain structures as well, so the neural events critical to the formation of CTA could interact in these regions. However, specific interconnections important to such interaction have not been identified. This area of the PCRF also is the general region identified as a vomiting trigger zone (Borison and Wang, 1949; see also Miller and Wilson, 1983). Thus, neural pathways or structures important to both CTA and vomiting could coexist in this general region. Whether common neural pathways or a discrete nuclear group of cells co-ordinating these two responses to motion exist remains to be demonstrated. Both responses are complex, involving many muscular events, and it may not be possible to identify a 'neural center' co-ordinating such responses. However, multidisciplinary research employing present technology for immunohistochemistry, electron microscopy, electrophysiology, biochemistry, and neuroanatomy portends the opening of new vistas for the understanding of the neural events underlying these behaviors.

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EXPERIMENTAL STUDIES OF GASTRIC DYSFUNCTION IN MOTION SICKNESS: THE EFFECT OF GASTRIC AND VESTIBULAR STIMULATION ON THE VAGAL AND SPLANCHNIC GASTRIC EFFERENTS

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Abstract

The experiments were conducted in anaesthetized rats. In the first part of the experiments, the effect of $CuSO_4$ on the afferent activity in the gastric branch of the vagus nerve was investigated. Gastric perfusion of $CuSO_4$ solution (0.04% and. 0.08%) provoked an increase in afferent activity. In the second part of the experiments, the reflex effects of gastric perfusion of $CuSO_4$ solution, repetitive stimulation of the gastric vagus nerve, and caloric stimulation of the right vestibular apparatus (5-18°C water) on gastric autonomic outflow were investigated. The results of these experiments showed that these three different types of stimulation caused an inhibition in efferent activity of the gastric vagus nerve and a slight activation of the splanchnic gastric efferents. The summation of the effect of each stimulation was also observed. These results, therefore, provide evidence for a possible integrative inhibitory function of the vagal gastric center as well as an excitatory function of gastric sympathetic motoneurons in relation to motion sickness.

Introduction

It has been generally recognized that nausea and emesis with gastric dysfunction are the main symptoms of space and motion sickness. It is assumed that vestibular as well as gastric

Keywords: gastric afferents, gastric efferents, vestibulo-vagal reflex, vestibulo-sympathetic reflex, gastrosensory-vestibular-autonomic interactions

stimulation can be the major sources of these symptoms. It is also well known that caloric stimulation of the vestibular apparatus can cause emesis and nystagmic responses. Wang and Borrison (1951) reported that the intragastric administration of copper sulfate induced emetic responses, and that the surgical interruption of the vagi had a more profound effect on the threshold and latency of vomiting than did sympathectomy, which caused no remarkable changes in these parameters. They stressed that the vagal gastric afferents play a more important role than splanchnic gastric afferents in the mediation of the gastric effects of copper sulfate. The present experiments were designed to study the effects of individual and combined vestibular and gastric stimulation on the reflex change in gastric autonomic outflow. Portions of the data describing the effects of copper sulfate on the rate of afferent discharges in the gastric branch of the vagus nerve have been reported elsewhere (Niijima *et al.*, 1987).

Methods

Male Wistar rats weighing 300–400 g were used. Food, but not water, was removed 5 hours before the experiment. Rats were anesthetized with 700 mg/kg of urethane and 50 mg/kg of chloralose, given i.p. A tracheal cannula was inserted.

The stomach could be perfused with copper sulfate ($CuSO_4$) or physiological saline through a catheter which was placed in the oesophagus and directed toward the cardiac portion of the stomach. Another catheter was placed in the pyloric portion of the stomach through the duodenum as an outlet for the perfusate.

Before starting the experimental perfusion, the stomach was washed with isotonic saline. Copper sulfate solutions (0.04% and 0.08%) and isotonic saline were used for the experimental perfusions. For each perfusion 4 ml of solution at 38°C were injected by syringe into the stomach over a 1-min period. The solution was kept in the stomach for 5-30 min, after which time the stomach was flushed for 1 min with isotonic saline. To stimulate the vestibular apparatus, the right external auditory meatus was irrigated for 3-10 min with cold water (5-18°C) and then flushed with warm water (34-35°C).

Afferent nerve activity was recorded from a nerve filament isolated from the peripheral cut end of the gastric branch of the vagus nerve, or of the splanchnic nerve. Efferent nerve activity was made from a filament isolated from the central cut end of the ventral gastric branch of the vagus nerve or the gastric branch of the splanchnic nerve. Nerve activity was amplified by means of a condenser-coupled differential amplifier, and stored on magnetic tape. Analysis of the nerve activity was performed after conversion of raw data to standard pulses by a window discriminator that distinguished the nerve discharges from the background noise. To monitor the time course of changes in neural activity the rate of neural discharge was determined by a ratemeter with a reset time of 5 sec. The output of this ratemeter was displayed on a pen recorder. Normal animal body temperature was maintained by means of a heating pad. The ECG was monitored throughout the experiment.

Results and Discussion

The Effect of Copper Sulfate on the Afferent Activity of the Gastric Branches of the Vagus Nerve

The perfusion of 4 ml of two different concentrations (0.04% and 0.08%) of $CuSO_4$ solution provoked an increase in afferent activity of the gastric branch of the vagus nerve (Niijima *et al*, 1987). After the onset of the perfusion with $CuSO_4$ the activity increased gradually and the increase lasted until after flushing of the gastric canal with isotonic saline. The stimulating effect of 0.08% solution of $CuSO_4$ was stronger than that of the 0.04% solution, and lasted for a longer period of time, as shown in the upper trace of Figure 1. With

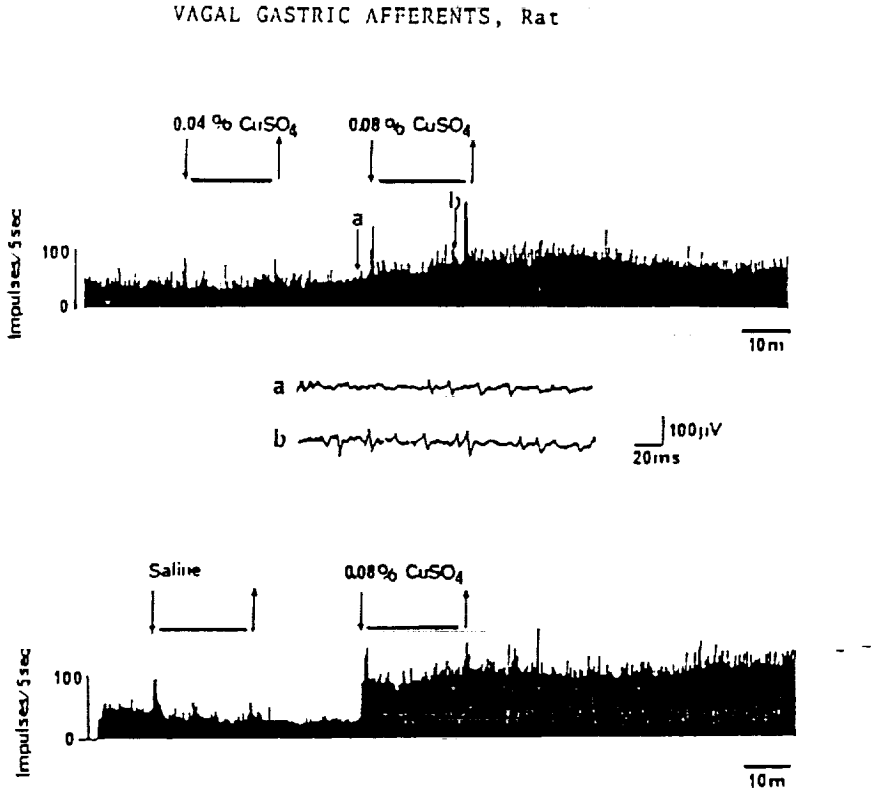

Fig. 1. Effect of gastric perfusion by 0.04% and 0.08% $CuSO_4$ solution and physiological saline on the afferent discharge rate of a vagal gastric nerve filament (from Niijima *et al.*, 1987). Downward arrows show time of onset of perfusion. Upward arrows show the end of rinsing with saline. Horizontal bars indicate the duration of perfusion with $CuSO_4$ solution and physiological saline. (*a*): sample of nerve activity taken at time indicated by arrow a, before perfusion with 0.08% $CuSO_4$;.(*b*): sample of nerve activity obtained at time indicated by arrow b, during perfusion with 0.08% of $CuSO_4$.

the 0.08% solution the increase in vagal activity lasted in general for more than 1 hour, even though the stomach was flushed after 20 min of exposure to the $CuSO_4$. The peak of activity provoked by the $CuSO_4$ was reached after perfusate had been flushed out of the stomach (Fig. 1, upper and lower trace). It is unlikely that these changes in neural activity resulted from mechanical effects of the infusion of solution into the stomach, because the perfusion of 4 ml of saline resulted in no noticeable change in discharge rate beyond the transient increase that was observed at the onset of perfusion and flushing of $CuSO_4$ solutions and saline (Fig. 1, lower trace).

Figure 2 shows the mean discharge rate in spikes/sec of five different preparations just before (control), 20 min after the onset of 0.08% $CuSO_4$ solution, and 30 min after flushing with saline. Those discharge rates are 6.4 ± 0.3 (S.E.M.), 13.4 ± 1.8 (S.E.M.) and 18.8 ± 2.3 (S.E.M.) respectively. The difference between firing rates obtained during the control period and the period 20 min after onset of perfusion, as well as between the control period and the period 30 min after flushing were statistically significant (Student's t-test).

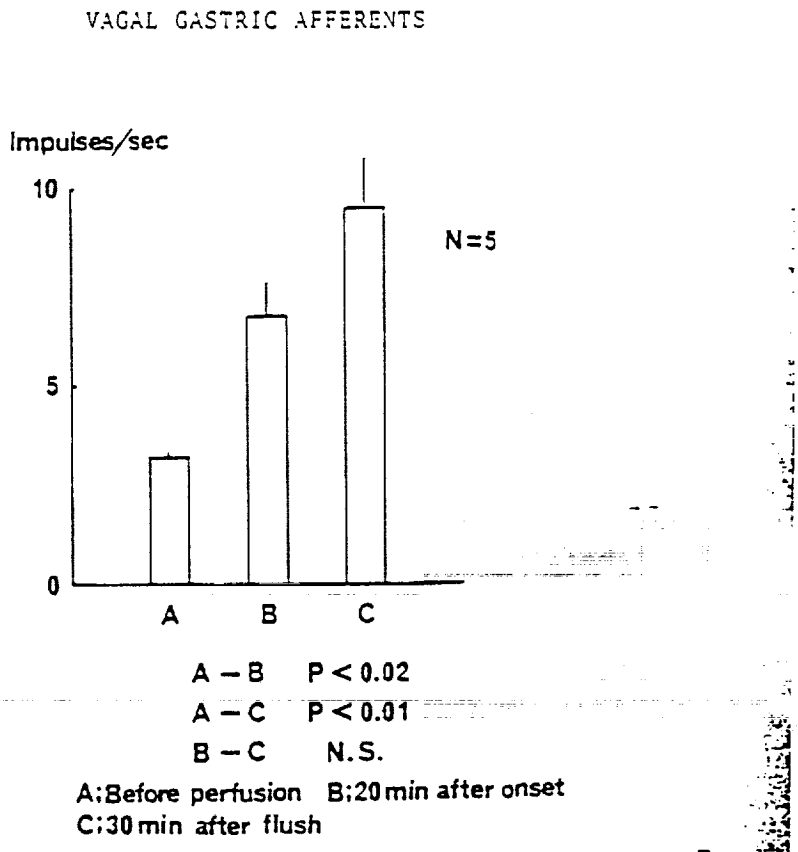

Fig. 2. Mean discharge rate of the gastric vagal afferents before A, 20 min after B and 30 min after rinsing C of perfusion by 0.08% $CuSO_4$ solution. (From Niijima *et al.*, 1987.)

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The Effect of Copper Sulfate on the Afferent Activity of the Gastric Branches of the Splanchnic Nerve

Figure 3 shows a typical change in the discharge rate of afferent fibers of the gastric branch of the splanchnic nerve. Except for the transient increases at the time of the onset of perfusion and rinsing, no remarkable change was found in the rate of afferent discharge during perfusion with the 0.08% $CuSO_4$ solution for 30 min or after flushing out by saline. These effects, in combination with those reported in the preceding section, indicate that the gastric effects of $CuSO_4$ were mainly mediated through gastric vagal afferents but that splanchnic afferent activity was not greatly altered by these stimuli.

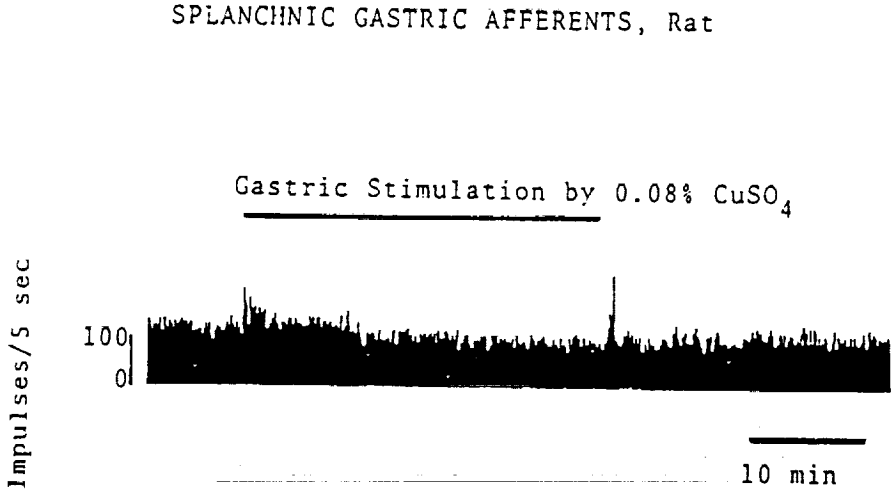

Fig. 3. Effect of gastric perfusion by 0.08% $CuSO_4$ solution on the afferent discharge rate of a splanchnic gastric nerve filament. Horizontal bar indicates the duration of perfusion with 0.08% solution.

It was established by Wang and Borison (1951) that the effective emetic concentration of $CuSO_4$ for oral administration was 0.08% in the dog and cat. The effect of intragastric $CuSO_4$ on the firing rate of gastric afferents is consistent with this in that the 0.08% solution produced a larger and more reliable change in the rate of firing than that produced by the 0.04% solution.

The specific receptors mediating the gastric vagal afferent response to $CuSO_4$ have not yet been identified, although several candidates exist. Mei (1985) has demonstrated the existence of vagal chemoreceptors in the intestinal wall, while Iggo (1957) has suggested that gastric pH receptors exist. Mei (1970) has also reported the existence of receptors in the mucous membrane of the gastrointestinal wall. While any of these receptors might be stimulated by $CuSO_4$ solutions, the exact source of the stimulating effect of $CuSO_4$ on gastric vagal afferents is not known.

The Effect of Caloric Stimulation of Vestibular Apparatus and Gastric Stimulation by Copper Sulfate on the Activity of the Vagal Gastric Efferent Nerve Fibers

As caloric stimulation of the vestibular apparatus, and gastric stimulation by $CuSO_4$ can cause the vomiting response in man (Wang and Borrison, 1951; Mano *et al*, 1988), a change in efferent activity in the vagal gastric nerve by these stimuli can be expected. Recordings of the efferent discharges were made from a ventral gastric branch of the vagus nerve.

The upper trace of Figure 4 shows the effect of caloric stimulation of the right vestibular apparatus on the rate of efferent discharges in the vagal gastric nerve. An application of cold water (10°C) on the right external meatus for 3 min caused a clear suppression in the rate of efferent discharge. The suppression continued even after the flushing of the meatus with warm water (34-35°C). It lasted about 17 min after cessation of the cold stimulation. A nadir was reached about 15 min after the onset of cold stimulation in this particular experiment. The lower trace of Figure 4 shows the effect of gastric stimulation by $CuSO_4$ and that of caloric

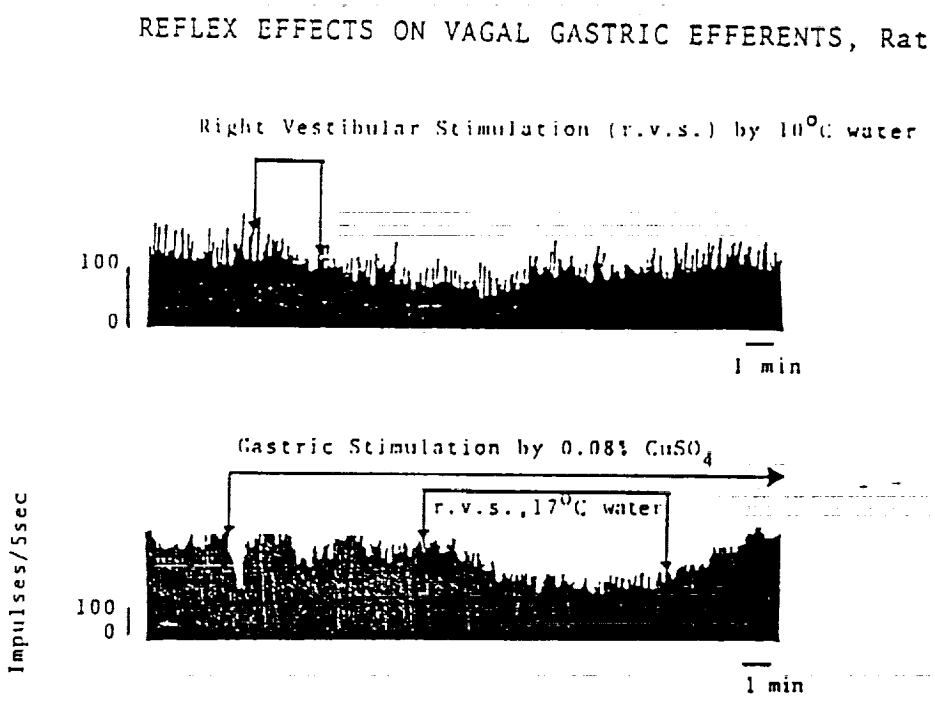

Fig. 4. Effects of gastric perfusion with 0.08% $CuSO_4$ solution and caloric stimulation of the right vestibular apparatus on the efferent discharge rate of the gastric branch of the vagus nerve. Vertical arrows in upper trace show time of onset and end of caloric stimulation of the right vestibular apparatus. First vertical arrow in lower trace indicates time of onset of gastric perfusion with 0.08% $CuSO_4$ solution. Second and third arrows show time of onset and end of caloric stimulation. Horizontal arrow shows duration of gastric perfusion.

stimulation on the vagal gastric efferent activity. At first, gastric stimulation with 0.08% $CuSO_4$ was applied, which caused a wave-like suppression in vagal activity. About 8 min after the onset of gastric stimulation, caloric stimulation of the right vestibular apparatus with 17°C water was applied for 11 min. This caloric stimulation caused a further stronger suppression in discharge rate. A nadir of suppression was reached about 7 min after the onset of caloric stimulation. As observed in the trace, the effects of gastric stimulation and caloric stimulation appeared to summate, and the effect of caloric stimulation was apparently stronger than that of gastric stimulation. Observations from two other preparations were consistent with the results. No remarkable suppressive response was elicited by gastric stimulation with 2% $CuSO_4$ (Fig. 5).

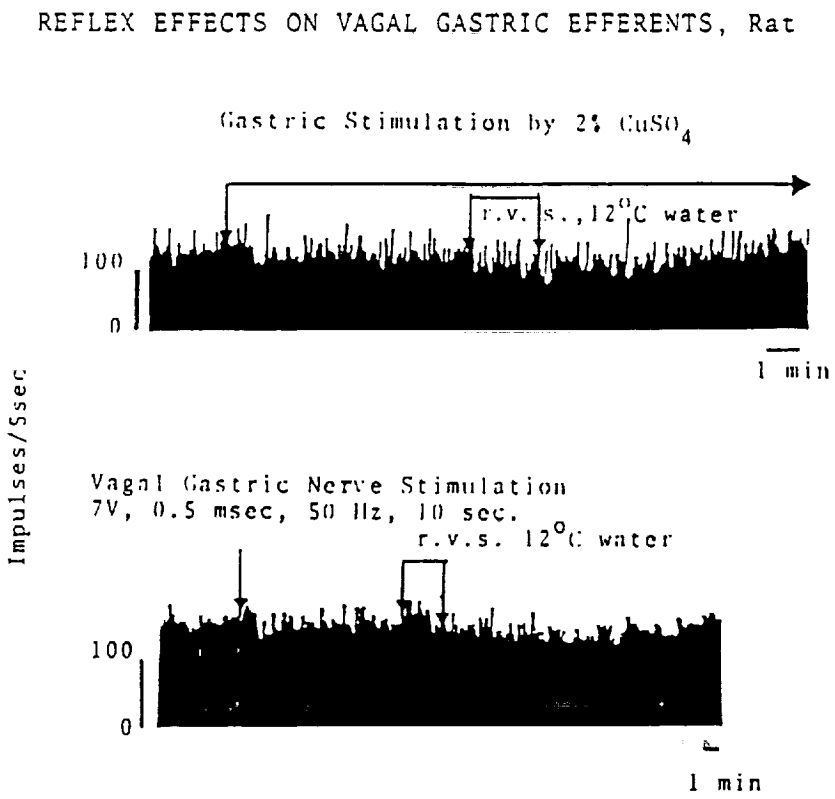

Fig. 5. Effects of gastric perfusion with 2% $CuSO_4$ solution, repetitive electrical stimulation of the gastric vagus nerve and caloric stimulation of the right vestibular apparatus on the efferent discharge rate of the gastric branch of the vagus nerve. Vertical arrows in upper trace indicate time of onset of gastric perfusion with 2% $CuSO_4$, and time of onset and end of caloric stimulation. Horizontal arrow indicates the duration of the gastric perfusion. First vertical arrow in lower trace shows time of electrical stimulation of the gastric branch of the vagus nerve, and second and third arrows indicate time of onset and end of caloric stimulation.

The lower trace of Figure 5 shows the effect of electrical stimulation of the gastric vagus nerve on the activity of the gastric vagal efferents. Two branches of the ventral gastric vagus nerve were used after sectioning. A pair of stimulation electrodes were placed on the central cut end of one branch, and recordings were made from the nerve filament dissected from the central cut end of another branch. As shown in the trace, a repetitive stimulation (7 V, 0.5 msec, 50 Hz for 10 sec) caused a long lasting inhibition in the rate of efferent discharge, lasting about 12 min. Caloric stimulation (12°C water) of the right vestibular apparatus for 3 min also resulted in a suppression lasting approximately 20 min.

The Effects of Caloric Stimulation of the Vestibular Apparatus and Gastric Stimulation by Copper Sulfate on the Activity of the Splanchnic Gastric Efferent Nerve Fibers

The top trace of Figure 6 shows the effects of gastric stimulation, as well as caloric stimulation of the right vestibular apparatus, on the efferent discharge rate of the gastric splanchnic nerve. These two stimulations for 5 min resulted in a slight facilitation in efferent discharge activity. The middle and lower traces show the effects of caloric stimulation for 5 min in different preparations. Caloric stimulation with water (5°C) of the right vestibular apparatus caused slight acceleration in splanchnic nerve activity in these two preparations.

These observations indicate that caloric stimulation of the vestibular apparatus as well as gastric stimulation with $CuSO_4$, resulted in a slight facilitation of gastric splanchnic efferent nerve activity.

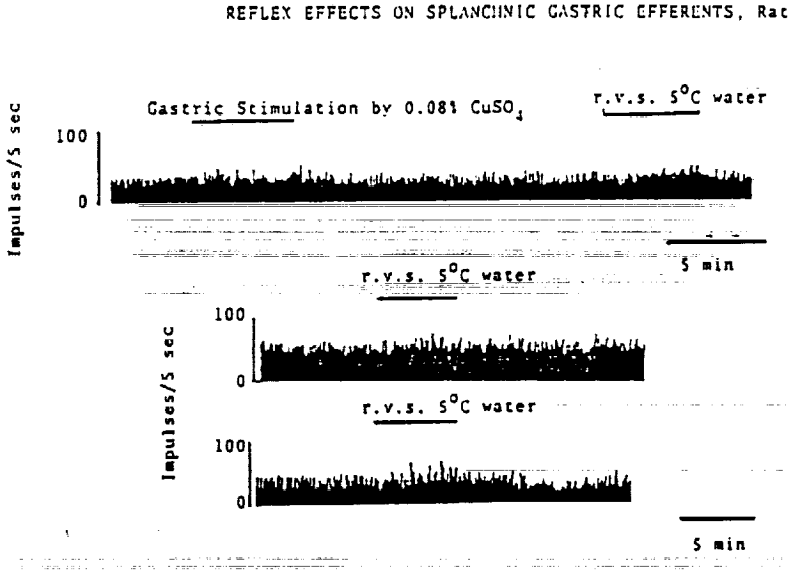

Fig. 6. Effects of gastric perfusion with 0.08% $CuSO_4$ solution and caloric stimulation of the right vestibular apparatus on the efferent discharge rate of the gastric branch of the splanchnic nerve. First horizontal bar on the top trace indicates time of gastric perfusion and second bar shows time of caloric stimulation. Horizontal bars on the middle and lower traces indicate time of caloric stimulations.

The results of these experiments can be summarized as follows: the different types of stimulation, such as gastric stimulation by $CuSO_4$, repetitive stimulation of the gastric vagus nerve and caloric stimulation of vestibular apparatus, caused an inhibition in efferent activity of the gastric vagus nerve and a slight activation of the splanchnic gastric efferents. This report therefore, provides evidence for a possible integrative inhibitory function of the vagal gastric center as well as a possible excitatory function of the gastric sympathetic motoneurons, which may play a role in space and motion sickness (Fig. 7).

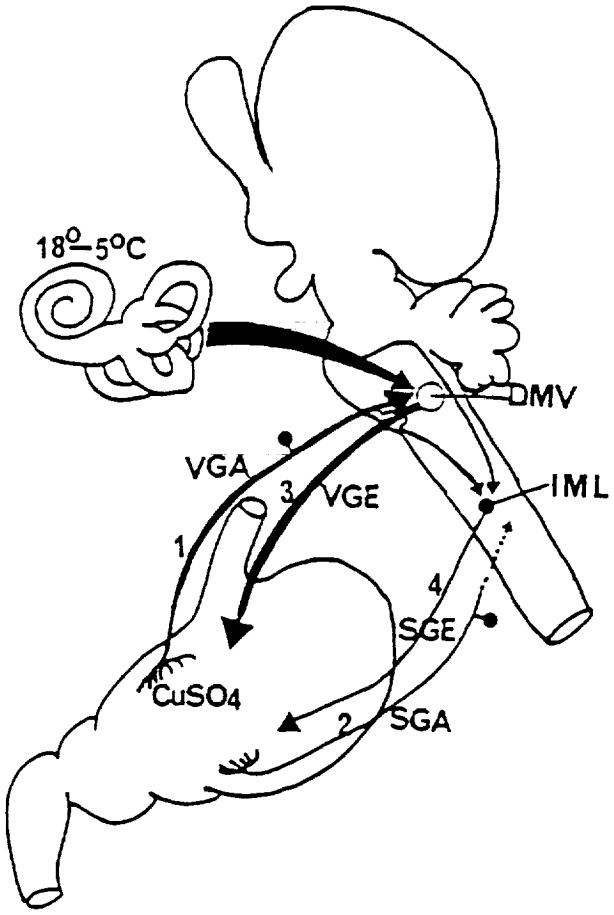

Fig. 7. Schematic illustration of the effects of gastric and vestibular stimulations on the activities of vagal and splanchnic gastric efferent outflows. *VGA*, vagal gastric afferents; *VGE*, vagal gastric efferents; *SGE*, splanchnic gastric efferents; *SGA*, splanchnic gastric afferents; *DMV*, dorsal motor nucleus of the vagus; *IML*, intermediolateral cell column (sympathetic preganglionic neuron group); *Inh.*, inhibition; *Exc.*, excitation.

Wang and Borrison (1951) reported that complete blockage of the emetic response to intragastric $CuSO_4$ required vagotomy combined with sympathectomy, and further suggested that the gastric splanchnic afferent pathway may play a role in the emetic response. However, our observations indicate that the chemical effect of gastric stimulation by $CuSO_4$ is not mediated by the gastric splanchnic afferents but by the gastric vagal afferents. It is suggested that the effects of mechanical stimulation such as distension of the gastric wall, can be mediated by the gastric splanchnic afferents and may play some role in the emetic response.

In relation to our observation of an increase in gastric sympathetic outflow following caloric stimulation of the vestibular apparatus, Mano *et al.* (1988) reported an increase in muscle sympathetic nerve activity (MSA) to the gastrocnemius-soleus muscle due to caloric stimulation of the vestibular apparatus in man. These findings may suggest the general activation of the sympathetic system and inhibition of the parasympathetic system in space motion sickness (however, see Akert and Gernandt, 1962; Megirian and Manning, 1967; Uchino *et al.* 1970, for reports of an opposite effect of vestibular stimulation).

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The Susceptibility of Rhesus Monkeys to Motion Sickness

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CORCORAN ML, FOX RA, DAUNTON NG. *The susceptibility of rhesus monkeys to motion sickness.* Aviat. Space Environ. Med. 1990; 61:807–9.

The susceptibility of rhesus monkeys to motion sickness was investigated using test conditions that are provocative for eliciting motion sickness in squirrel monkeys. Ten male rhesus monkeys and ten male Bolivian squirrel monkeys were rotated in the vertical axis at 150°/s for a maximum duration of 45 min. Each animal was tested in two conditions, continuous rotation and intermittent rotation. None of the rhesus monkeys vomited during the motion tests but all of the squirrel monkeys did. Differences were observed between the species in the amount of activity that occurred during motion tests, with the squirrel monkeys being significantly more active than the rhesus monkeys. These results, while substantiating anecdotal reports of the resistance of rhesus monkeys to motion sickness, should be interpreted with caution because of the documented differences that exist between various species with regard to stimuli that are provocative for eliciting motion sickness.

THE RHESUS MONKEY has not been considered a good model for motion sickness research because these animals are thought to be resistant to motion sickness (1,16). However, the resistance of rhesus monkeys to motion sickness has not been clearly documented and most of the available information on motion sickness susceptibility in these animals is anecdotal (personal communications G. H. Crampton and J. Lackner).

Apparently, the evaluations of motion sickness susceptibility in rhesus monkeys have been opportunistic rather than by design. For this reason parameters known to be important for inducing motion sickness in squirrel monkeys and other susceptible species (3,4,5,9,18) have not been examined systematically in the rhesus monkey. For instance, it is well known that head movements out of the plane of rotation produce cross-coupled accelerations which affect the efficacy of rotation as a motion sickness stimulus (15). Thus, the susceptibility of squirrel monkeys to rotation is greatly reduced when they are restrained in primate chairs (5). Similarly, head stabilization prevents or greatly reduces motion sickness in these animals (18) as well as in humans (9). While it is impossible to determine the degree to which movement was allowed in some previous investigations using rhesus monkeys, in other studies it is apparent that voluntary movements were restricted because the animals were restrained in primate chairs during testing. Therefore, previous tests of susceptibility of rhesus monkeys to motion sickness may have produced negative results due to test conditions that were inadequately provocative, rather than to the resistance of rhesus monkeys to motion sickness.

This investigation was undertaken to compare the motion sickness responses of the rhesus monkey with those of the squirrel monkey, using stimuli known to elicit motion sickness in the majority of squirrel monkeys. In all tests unrestrained voluntary movement was permitted within a test cage large enough to allow head and whole-body movements of sufficient magnitude to produce cross-coupled accelerations.

METHODS

Subjects: Ten sub-adult (approximately 4 years of age) male rhesus monkeys (*Macaca mulatta*) with no previous history of motion testing, and ten adult (approximately 7 to 10+ years of age) male Bolivian squirrel monkeys (*Saimiri sciureus*) selected randomly from a pool of animals used previously in motion sickness studies, were tested. The animals were housed in standard colony conditions and were maintained on 12:12 (rhesus monkey) and 14:10 (squirrel monkey) light:dark cycles. Food and water were available *ad lib*. On each test day the monkeys were fed fresh bananas in the experimental chamber approximately 5 min before testing began.

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The experiments were conducted at the Ames Research Center and conformed to the Center's requirements for the care and use of animals.

Apparatus: Rhesus monkeys were exposed individually to rotation while free to move about in a stainless steel cage (60 × 60 × 60 cm) that was mounted on a Goerz Model 611 turntable. The lower half of three walls (30 cm) of the cage was formed by solid stainless steel panels, while the ceiling and the upper half of the cage were made of wire mesh. One wall of the cage had a guillotine door made of clear Plexiglas.

Squirrel monkeys were exposed individually to rotation, while free to move in a cage constructed of clear Plexiglas (52 × 23 × 30 cm) that was mounted on the turntable. To make the conditions of visual stimulation comparable for squirrel and rhesus monkeys, aluminum foil was attached to the lower half (15 cm) of three walls of this cage to simulate the visual conditions formed by the stainless steel panels of the cage used for the rhesus monkeys.

During rotation both the rhesus and squirrel monkeys could view the lighted test chamber by looking directly out through the clear walls of the cages while sitting upright, or by looking up through the upper portion of the walls or the ceiling, if in a crouched or prone position. The animals could see through the door of the cages from floor to ceiling when oriented in that direction.

Procedure: Two conditions of vertical-axis rotation were used. In the first test, "Continuous Rotation," the animals were exposed to clockwise rotation at 150°/s for a maximum duration of 45 min. In the second test, "Sudden-Stop," the animals were exposed to periods of rotation alternating with brief periods during which the cage was stopped. The second test was conducted 1.5–2 months after the first motion test. A velocity-ramp was used to drive the turntable in this Sudden-Stop Condition. The cage was accelerated at 75°/s^2 for 2.0 s to reach the rotation velocity of 150°/s, then maintained at constant velocity for 23.6 s, and then decelerated at 88°/s^2 for 1.7 s to 0°/s (stopped). The turntable remained stationary (stopped) for 3.1 s. This alternation of rotation with stationary periods continued for a maximum duration of 45 min (90 cycles). The direction of rotation was clockwise for the first 30 min and counterclockwise for the remaining 15 min of exposure. If an animal vomited, motion was terminated 5 min after the first vomiting episode.

The animals were observed continuously during tests, and latencies to retching and vomiting were recorded. To characterize the activity level of animals during the Sudden-Stop Condition, the duration (in s) of periods of inactivity was recorded on a printout counter using a manual switch operated by an observer. A state of "inactivity" was defined as a 5-s period during which neither head movements nor whole-body movements of the animals were observed. Small arm movements which did not affect head or whole-body orientation were ignored.

RESULTS

None (0/10) of the rhesus monkeys retched or vomited during either condition of rotation, but all (10/10) of the squirrel monkeys retched and vomited during both conditions. For the squirrel monkeys the mean latency to the first vomiting episode was significantly shorter during continuous rotation (3.6 ± 2.0 min) than during intermittent rotation (7.8 ± 3.0 min) [$t(9) = 6.87$, $p < .001$].

Voluntary movements made during the motion tests differed greatly, with some animals moving continuously and others remaining still for extensive periods. The amount of activity that occurred during the motion tests varied more among the rhesus than among the squirrel monkeys. The percentage of the test session during which the individual rhesus monkeys were active ranged from 20% to 92%, while the percentage of active time ranged from 79% to 100% for the squirrel monkeys. The squirrel monkeys were active a greater percentage of the time (median = 100%) than the rhesus monkeys (median = 77%), Mann-Whitney $U = 5$, $p < 0.02$. Of the 10 rhesus monkeys, 6 were less active than all of the squirrel monkeys and 5 of the 10 squirrel monkeys had no periods of inactivity (i.e., were active 100% of the time).

DISCUSSION

Although it has been shown that rhesus monkeys have a complete emetic reflex (6,8,11,12,14,17,19), there appears to be no published evidence documenting their responses to motion stimuli. The results of this study indicate that they apparently are not susceptible to motion sickness during either continuous or intermittent vertical-axis rotation, stimuli that elicit motion sickness in squirrel monkeys (2,5), chimpanzees (13), and humans (7,10). Although the influence of age on susceptibility to motion sickness has not been rigorously investigated, studies with rats, squirrel monkeys, and humans (2) suggest that susceptibility to motion sickness may decrease with advancing age. This information indicates young, or sub-adult animals may be the most susceptible of the species. If this is correct, the rhesus monkeys tested in this experiment should have biased results toward detecting motion sickness in the rhesus monkey. The fact that none of the rhesus vomited in this study substantiates anecdotal reports and is consistent with unpublished comments that they are refractory to motion sickness.

However, several factors indicate that caution should be exercised in concluding that rhesus monkeys are immune to motion sickness. Motion sickness is known to be elicited most effectively by qualitatively different stimuli in various susceptible species (2). One example is the differential susceptibility of the squirrel monkey and the cat to vertical-axis rotation and vertical-linear acceleration. While the squirrel monkey is highly susceptible to rotation but not to vertical bouncing, the reverse is true for the cat. Such differential susceptibility of species to selected motion profiles reveals how difficult it is to demonstrate complete immunity to motion sickness. This fact indicates that additional testing with stimuli of different types (i.e., linear acceleration, parallel swing, etc.) should be conducted before concluding that rhesus monkeys cannot be made motion sick.

Evaluation of observational data also suggests that

caution should be exercised before concluding that rhesus monkeys are not susceptible to motion sickness. When the animals in this study were tested using continuous rotation at 150°/s, it appeared that the activity levels of the two species were quite different, with the squirrel monkeys being more active during rotation. The lower level of activity of some rhesus monkeys could perhaps be interpreted as a "behavioral strategy" to minimize vestibular stimulation during rotation. Two characteristic behavioral patterns occurred in the rhesus monkeys: (a) Rhesus monkeys that were active during rotation periodically terminated movements and adopted positions which stabilized their heads and/or bodies. Such positions included leaning against the wall, placing the jaw or head against the wall, or lying prone on the floor of the cage. (b) Rhesus monkeys that were characteristically inactive during rotation commonly adopted a prone position on the floor of the cage, often with their heads very close to the axis of rotation. In addition, informal observations of the squirrel and rhesus monkeys indicated that their spontaneous movements were distinctly different. The squirrel monkeys made many high-frequency, jerky movements, with numerous small pitching movements of the head, while the rhesus monkeys tended to sit upright and made relatively slower head and body movements in the yaw plane with fewer, and slower pitch movements. Thus, inherent behavioral differences between these two species could lead to qualitative differences in actual stimulation resulting from a single imposed stimulus condition (e.g., rotation).

These observations suggest that rhesus monkeys might behave in a manner that minimizes vestibular stimulation produced by continuous vertical-axis rotation and thereby avoid becoming motion sick. Therefore, to ensure that the animals were subjected to angular accelerations even if they were inactive, the Sudden-Stop Condition was used. However, during the Sudden-Stop Condition vomiting latencies increased for the squirrel monkeys indicating that, at least for squirrel monkeys, this stimulus was less provocative than continuous rotation. Thus, the Sudden-Stop Condition may not have been a more provocative stimulus than continuous rotation, and therefore, may not have been a stringent test of susceptibility for the rhesus monkeys.

The test conditions used in this experiment do not provide a comprehensive evaluation of the susceptibility of rhesus monkeys to motion sickness. Further testing with a wider range of motion stimuli should be considered. If other tests continue to indicate rhesus monkeys are immune to motion sickness, then comparative investigations of neural, physiological, and hormonal differences between rhesus monkeys and other primates susceptible to motion sickness may be a useful approach to increase our understanding of the mechanisms underlying the etiology of motion sickness.

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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

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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)

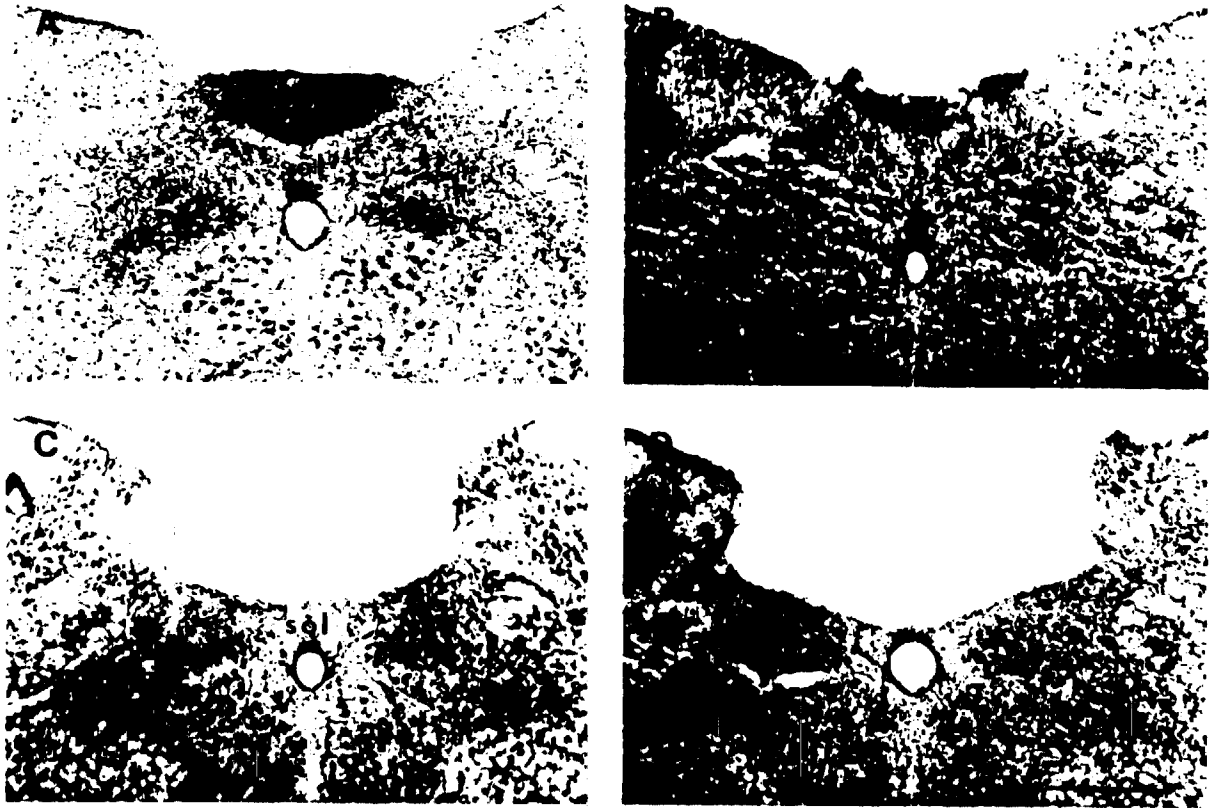

FIG. 1. Coronal sections through the brainstem at the level of the obex showing (A) the AP from a rat in the sham-operated control group; (B) a representative section from an animal with sparing of the rostral AP (lesion rating of 2); (C) an example of a lesion incorporating the AP and subpostrema (lesion 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.

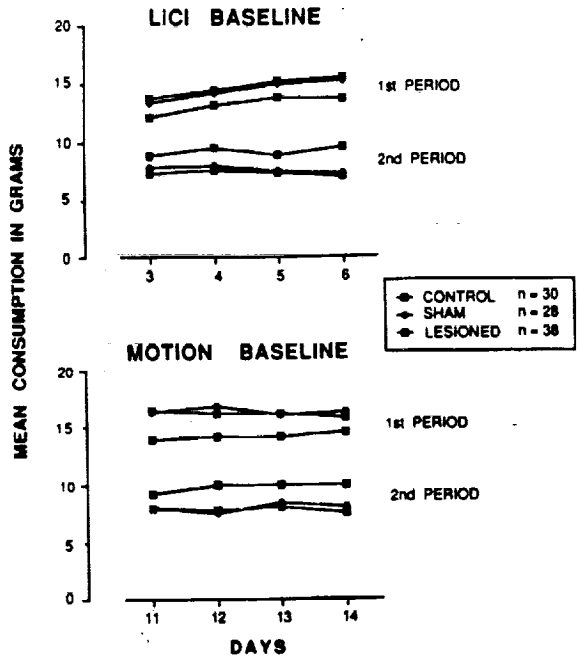

FIG. 2. Mean tap water consumption for animals in the three lesion conditions during the last 4 days of adaptation to limited water access prior to conditioning with LiCl (upper panel) and prior to conditioning with motion (lower panel). The average intake of tap water for each lesion group is shown 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

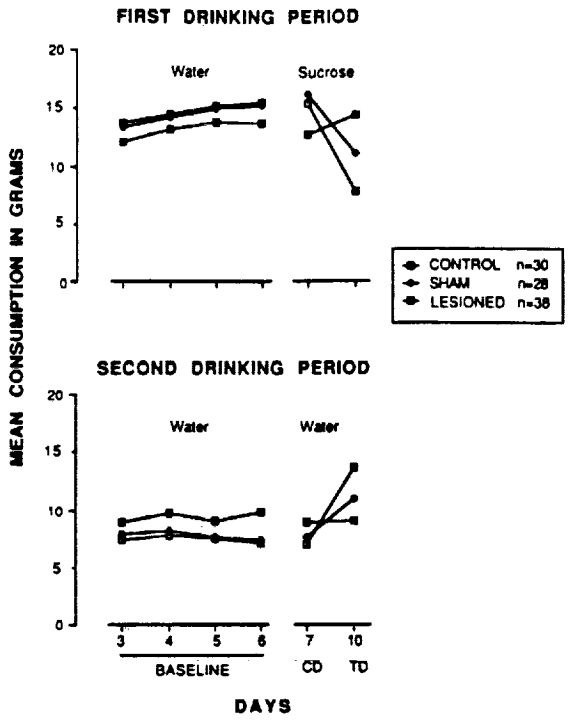

FIG. 3. Mean fluid consumption for animals in the three lesion conditions in both drinking periods of the LiCl conditioning experiment. The data on the left of each panel (reproduced from Fig. 2) represent intake of tap water over the last 4 days of the 6-day adaptation to limited water access during 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.

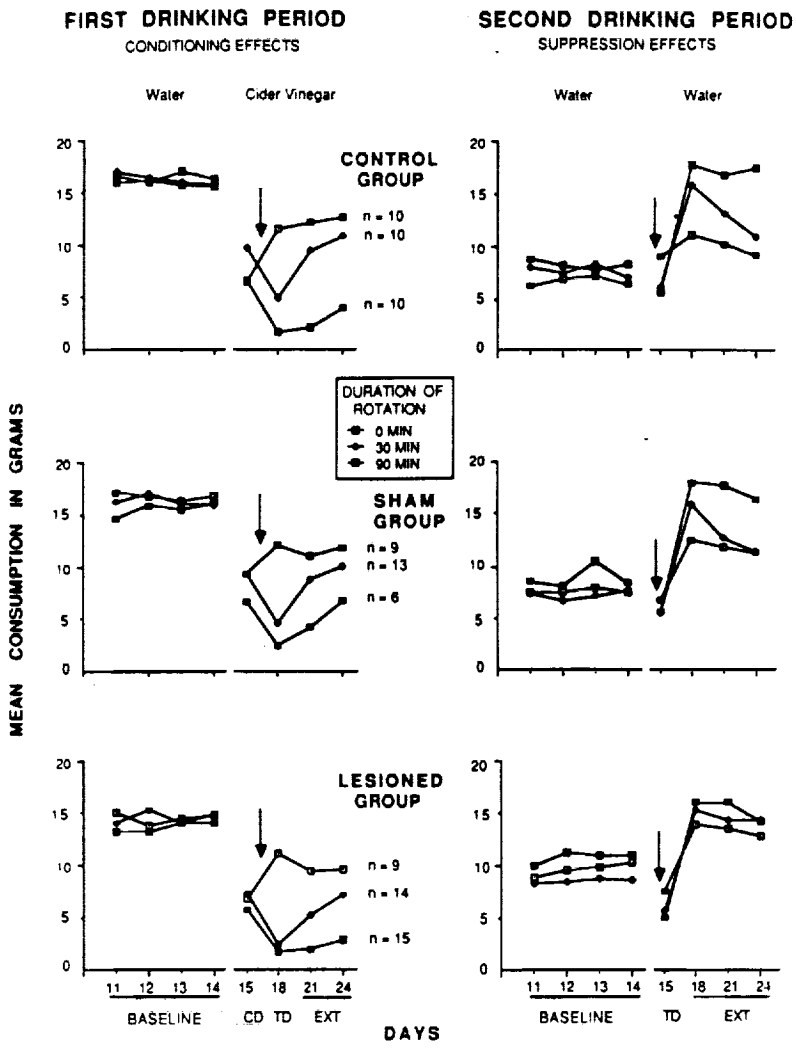

FIG. 4. Mean fluid consumption during both 10-min drinking periods by animals in the nine groups of the rotation conditioning experiment. Data in the left column represent baseline water intake over the 4 days between the LiCl experiment and conditioning with rotation and the cider vinegar intake on 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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Chapter 8

INVESTIGATING MOTION SICKNESS USING THE CONDITIONED TASTE AVERSION PARADIGM

Robert A. Fox

TABLE OF CONTENTS

I. INTRODUCTION

The avoidance of foods which are associated with uncomfortable or aversive internal states has long been recognized. Many people are aware, either directly or via anecdotal reports, of individuals who avoid foods which were eaten just before the onset of sickness. Awareness of this phenomenon can be traced to the writings of John Locke.[1] The disruption of diet during cancer therapy is sometimes ascribed to the attribution of an unpleasant quality to foods eaten preceding the sickness induced by therapy itself.[2] In addition, it has long been recognized by the manufacturers of rodent poisons that animals avoid the injection of food treated with nonlethal doses of poison.[3,4]

An important part of the laboratory study of this phenomenon has been directed toward studying the role learning plays in this type of avoidance behavior. Following the lead of Garcia and his associates, this avoidance has come to be interpreted as arising from a form of classical conditioning. In typical laboratory studies of this behavior, a novel food is ingested just prior to exposure to some stimulus, commonly poisoning or irradiation, which produces illness. Following the terminology of classical conditioning, it is common to describe this procedure as one of "pairing" a conditioned stimulus (CS), the novel food, with an unconditioned stimulus (US), the illness induced by toxicosis or irradiation. Avoidance of the food in succeeding feeding opportunities is viewed as a learned response or a conditioned taste aversion (CTA).

Garcia and associates have argued that this form of learning is biologically significant in that it serves to regulate the "internal milieu", presumably by adjusting the hedonic value of food via feedback from the viscera.[5,6] Work by Garcia and collaborators has generated considerable debate in the psychological literature regarding this form of conditioning and its impact on traditional theories of learning.[1,7] Various bibliographies[8,9] and reviews[10-12] dealing with these issues are available in the existing literature. These sources should be consulted for detailed discussion of theoretical issues.

The persistent conception that "illness", particularly visceral illness, serves as the US for the development of CTA is of more importance to the use of CTA in motion sickness research. Early studies of CTA typically used either exposure to irradiation or injection of toxins as the US. Most stimuli used as USs in these studies were known to produce sickness in the form of nausea or vomiting in humans or animals. Thus, the assertion that sickness produced by these treatments was the functional US producing the observed CTA was a natural inference. In addition, Garcia and Ervin[5] discussed the anatomical convergence of gustatory and visceral afferents in the nucleus of the solitary tract, and thus suggested a putative neural system to account for the unique propensity, demonstrated by Garcia and Koelling,[13] for gustatory stimuli to become associated with visceral disruption (i.e., "sickness").

Garcia et al.[14] asserted that motion sickness could produce "gustatory" aversions, but passive motion was first reported as an US to establish CTA by Green and Rachlin.[15] The purpose of this chapter is to review the manner in which CTA has been used to study motion sickness. Numerous reviews concentrating on other aspects of CTA are available in the existing literature. Readers are encouraged to consult the various papers[16-22] and edited books[11,23] for extensive information on other aspects of this literature.

II. RATIONALE FOR USING CTA

The assumption that an unspecified, aversive internal state resulting from exposure to passive motion is the effective stimulus producing CTA underlies the use of CTA to measure motion sickness. Various forms of evidence support the inference that CTA produced using passive motion as the US results from motion sickness. In early studies investigating the use of motion-induced CTA in rats it was noted that lithium chloride, cyclophosphamine, or irradiation have nauseogenic and emetic effects in other animals or in humans, and presumably produce general

malaise which serves as the US in rats. Because nausea and gastrointestinal distress are common components of the motion sickness syndrome, the inference that motion-induced CTA arises from illness is plausible. The presumed importance of an internal state, or illness, as the US was supported further by the demonstration that motion-induced CTA occurs more readily to gustatory cues than to either proprioceptive or exteroceptive cues.[24] This finding is consistent with data for poison-induced CTA[6] and lends plausibility to the inference that exposure to motion disrupts an internal state, thereby producing CTA. In addition, the vestibular system plays a critical role for the efficacy of motion as an US to produce CTA. After surgical damage to the vestibular system, motion-induced CTA is either prevented,[25] or greatly attenuated.[26] Thus, as motion sickness does not occur in labyrinthine-defective humans[27] or animals,[28,29] motion-induced CTA does not occur when the vestibular system is destroyed in rats.

Following this general conception of a plausible relationship between CTA and sickness, two principal applications have evolved for using CTA to assess motion sickness. The most prevalent is to view CTA as a behavioral reflection of motion sickness that may be useful with species such as the rat which are incapable of vomiting.[30] This concept was implied by Hutchison[31] and furthered by Mitchell and collegues[32,33] who showed that both pica and CTA could be produced by rotation and then argued that these two effects of rotation should be considered species-specific reactions to motion sickness. The second application is to assess subemetic symptoms of motion sickness in animals capable of vomiting. This application was suggested for squirrel monkeys by Roy and Brizzee,[34] and Wilpizeski and Lowry[35] have proposed a theory interpreting nausea as the US for CTA in squirrel monkeys.

III. GENERAL MODEL OF THE CTA PARADIGM

A. DEFINING CHARACTERISTICS

Various aspects of a general model of the CTA paradigm, with particular attention to factors which are important to the application of CTA to the study of motion sickness, are discussed in this section. This model has the following characteristics: (1) A flavored stimulus (often a novel fluid) serving as a CS is offered to the animal. (2) Some form of passive motion, most often involving rotation, is used as a US. Exposure to this motion typically occurs soon (within minutes) after access to the CS is withdrawn. (3) A period during which recovery from the direct effects of the US can occur (perhaps 2 or more days) follows the joint presentation (i.e., "pairing") of the CS and US. (4) The CS is presented by itself (an "extinction trial") to determine the strength of CTA developed by pairing the flavored stimulus with passive motion. Various modifications on this general model may occur for experimental reasons or because of limitations arising from practical considerations in a specific study.

B. A POTENTIAL ADVANTAGE OF CTA OVER OTHER DEPENDENT MEASURES

While vomiting is well defined and universally accepted as the endpoint of motion sickness, the identification of nausea, and the interpretation of the various other effects of motion which accompany motion sickness are less clear, particularly in animals. Of special interest here are the disorientation and disruption of locomotion and motor skill which may be produced by exposure to passive motion. Reason and Brand[27] referred to these accompanying effects as epiphenomena to the "big four" reactions of motion sickness: pallor, cold sweating, nausea, and vomiting. Other putative dependent measures of motion sickness, especially those which reflect sickness via reductions in behavior, are prone to influence by these accompanying effects of motion in addition to being influenced by motion sickness itself. These other measures include spontaneous activity,[36] operant responding for food reinforcement,[37] and fluid intake.[25] While these measures need not necessarily be affected by factors other than sickness,[38] each could be suppressed by accompanying effects and by various exteroceptive stimuli (i.e., noise, vibration, or other stimuli associated with the production of passive motion stimulation).

On the other hand, CTA is produced by exteroceptive cues only with difficulty. In addition, when CTA is used as the dependent variable for assessing motion sickness, disorientation, disruptions of locomotion, and other residual effects dissipate during the recovery period following the pairing of a CS with exposure to passive motion (see number 3 above). Thus, this recovery period between the conditioning and evaluation phases of the CTA paradigm serves to isolate the evaluation of motion sickness from various direct effects of motion which may not be the intended referent of "motion sickness". This characteristic of the CTA paradigm provides CTA with an advantage over some other putative measures of motion sickness. Changes in those putative measures which indicate sickness by increases in behavior (the intake of nonnutritive substances, or pica[32,33]) may be reduced by accompanying effects of motion, but these measures will not provide a false positive indication of sickness. Because the rate of defecation can be affected by general arousal, animals should be acclimated to the experimental conditions before testing.[39]

C. POTENTIAL CONFOUNDING VARIABLES IN CTA

1. Novelty and Salience of the Conditioned Stimulus

The relative novelty of a taste can have a profound influence on the strength of an aversion conditioned to that taste. It is generally the case that stronger aversions are formed to novel tastes than to familiar tastes.[40,41] However, CTAs can be formed to familiar tastes in animals,[42,43] in children,[44] and in adult humans.[2] Thus, it is not imperative that a novel taste be used as a CS. In many cases, particularly when rodents or other small laboratory-bred animals are used, the feeding history of subjects is controlled and known and a novel-flavored food or solution can be used to make a sensitive test for CTA. When primates such as the popular squirrel monkey are used, the selection of palatable, novel-flavored stimuli can be problematic. Caution should be used when pretesting a flavor to assess its palatability because pretesting itself may influence the effectiveness of that flavor as a CS. Exposure to a potential CS preceding conditioning clearly attenuates the strength of an aversion to that cue.[45,46]

The term salience has been used to describe the propensity of a cue to become conditioned. Rozin and Kalat[22] demonstrated that all tastes are not equally associable to the internal consequences of poisoning in rats. Those tastes to which stronger aversions were formed were referred to as more salient. The salience of a cue may be affected by its novelty, intensity, palatability, and intrinsic taste quality (see Kalat[47] for references). Rozin and Kalat[22] demonstrated that palatability order, determined as preference in choice tests, does not necessarily correspond to the salience order for a set of taste cues. Kalat[47] varied the concentration of flavored solutions used for conditioning to investigate the role familiarity may play in determining salience. For rats reared on tap water, the more concentrated of two solutions was associated better with illness. For rats reared on an even more concentrated solution, the less concentrated solution was associated better with illness. Kalat suggested that unfamiliarity (novelty) is a major determinant of salience. Salience also appears to be affected by cue characteristics other than taste alone. Solutions typically used in studies of CTA may differ in odor as well as in taste. By rendering rats anosmic, it has been shown that olfactory cues can combine with taste cues to increase the salience of a "flavored" (i.e., a taste) stimulus.[48]

The novelty and salience of cues used as CSs are clearly related to the strength of CTAs and could impact importantly on studies using CTA to investigate motion sickness. A research objective which requires repeated conditioning with a given animal will be influenced by these effects. The same cue should be used as a CS in successive conditioning attempts using different USs only with caution, and precise matching of cues for novelty/salience is very difficult and costly, if possible. Several investigations of the relative salience of some cues have been conducted for rats,[47-51] but similar studies for other species used in motion sickness research (i.e., dog, cat, and monkey) have not been conducted. Certainly, any comparison of the strength of CTA associated with different phases of an experiment must be made cautiously or avoided

completely. A design where a cue is presented repeatedly but an aversion is not formed simultaneously provides preexposure to the CS, which may reduce the strength of CTA conditioned to that cue later. Thus, the demonstration of CTA in later conditioning conservatively shows CTA can be produced, but it does not pose a sensitive test of the strength of that CTA. In addition, any design using repeated conditioning will also expose animals to aversive internal consequences, either from the same or another US, and such exposure can significantly attenuate the strength of CTAs induced later (see below).

Research investigating the effects of lesions on motion-induced CTA should consider effects of those lesions on salience as well as on the efficacy of the US (i.e., on motion sickness). It has been shown, for example, that lesions of area postrema influence food consumption in rats.[52] While the effects of lesions on other neural structures of interest to motion sickness research are not necessarily known, it should be recognized that surgical interventions could affect the magnitude of CTA by altering reactions of the animals to the CS as well as to the US or its internal effects.

2. Prior Exposure to the Unconditioned Stimulus

The strength of conditioned aversions generally is greatly reduced by exposing animals to an aversion-producing treatment prior to conditioning. That is, animals exposed to an aversion-producing treatment prior to the pairing of that, or a different treatment with a flavored food, commonly form aversions less readily than animals exposed to a control treatment prior to conditioning. In some cases this exposure before conditioning completely prevents the formation of a conditioned aversion. This effect can occur when any of various conditioning procedures and aversion-producing treatments are used.[53] The degree of reduction in magnitude of CTA which is produced by exposure to a treatment prior to conditioning generally increases as the number of exposures prior to conditioning increases, but reduced magnitude of conditioning has been demonstrated with a single exposure preceding conditioning.[53,54]

Braveman[53] referred to experiments in which animals are exposed to one potentially aversion-producing treatment and then conditioned with a different treatment as "crossover" experiments. Experiments of this type have been conducted to exclude addiction or tolerance to the drugs commonly used as aversion-producing treatments as explanatory factors for the effect. However, in a series of crossover experiments of particular importance to the use of CTA as a measure of motion sickness, Braveman[54] (Experiment 5) demonstrated that five exposures to doses of methylscopolamine, d-amphetamine sulfate, or lithium chloride prior to conditioning with motion blocked the formation of an aversion when rotation (60 rpm for 15 min) was used 5 d later as an US.

The blocking of motion-induced CTA by preconditioning exposure to aversion-inducing drugs is a finding of cardinal importance to the use of CTA in studies of motion sickness. Braveman suggested this blocking effect may depend on exposure to a treatment which can be used as an US for producing CTA. The existence of this effect dictates that CTA should not be used to measure motion sickness when animals have been exposed to any of the myriad of drugs known to be an effective US for CTA. This can be of special concern when primates, which are sometimes tested on several occasions over a period of years, are used in motion sickness research. A conservative interpretation would indicate that CTA should not be used, or at least should be used with caution, if animals have been tested previously with emetic drugs, or with other drugs such as scopolamine, which can be used as an US to produce CTA.

In addition, passive motion itself meets Braveman's criterion of being a treatment capable of producing CTA, and the attenuating effect of exposure to a treatment before conditioning is typically robust when animals are exposed to the identical treatment that is to be used for conditioning. Thus, it would appear that CTA might be expected to be weak when conditioning follows several exposures to the motion used later as an US. Harounian et al.[25] (Experiment 3b) reported that exposure to interrupted rotation before conditioning prevented the formation

of CTA in rats when that same motion was used as an US. In this experiment preconditioning exposures consisted of rotation of water-deprived rats on five separate occasions in order to study postrotational suppression of drinking. Exposure to rotation before conditioning clearly reduced the magnitude of CTA produced by later conditioning. All animals were exposed to motion the same number of times prior to conditioning, and the parameters of the motion (i.e., speed of rotation, etc.) were not varied. Thus, it is not possible to determine the minimum number of exposures to rotation which will produce this effect or whether this minimum number is affected by the type of passive motion that is used. It is clear from this experiment, however, that serious confounding of effects could arise if the number of exposures prior to conditioning varies for different animals. This potential problem should be considered if animals to be used in a conditioning study may have been used in previous motion sickness research.

3. Interaction Between Unconditioned Stimuli

The efficacy of an US can be influenced by other stimuli present at the time the US is applied. Electric shock typically is not an effective US for establishing CTA. However, Lasiter and Braun[56] have shown that rotation-induced CTA is enhanced when rats are exposed to footshock in conjunction with rotation. In a second experiment reported in this paper it was demonstrated that footshock also enhanced the magnitude of CTA produced using apomorphine as the US. Thus, it appears that the enhancing effect of footshock on rotation-induced aversion is not necessarily due to increased vestibular stimulation arising from movements elicited during rotation. The authors suggest this enhancement is due to increased arousal produced by the footshock. This demonstration of enhanced CTA by a stimulus which is not an effective US for CTA indicates that control groups should be included when the method of exposure to motion might affect the level of arousal of animals.

IV. IMPLEMENTATIONS OF THE PARADIGM

A. PASSIVE MOTION AS AN UNCONDITIONED STIMULUS

Simple, vertical axis rotation is the most common form of passive motion used to produce CTA. Rotation speeds range from as low as 12 rpm (72°/s) to as great as 198 rpm (1188°/s), but most studies have used speeds of 30 to 40 rpm (180 to 240°/s). Because the vestibular system is affected only by accelerations, precise specification of the parameters of motion which comprise the US is complicated when this type of stimulus is used. If animals are restrained and positioned so the vestibular system is directly over the axis of rotation, accelerations will occur only briefly at the beginning and ending of rotation. However, if voluntary movement is permitted during rotation, undefined accelerations are produced when the head is moved. Cross-coupled accelerations, which are especially provocative for producing motion sickness in man, occur if the head is moved in a plane differing from the plane of rotation. No studies requiring animals to make voluntary head movements producing such cross-coupled accelerations have been reported.

Several forms of passive motion have been used to ensure accelerations are applied to the vestibular system independently of voluntary movements made by the animals during rotation. A simple method for accomplishing this is to start and stop, i.e., to interrupt the motion. This method might be called interrupted vertical axis rotation. This form of rotation has been used in experiments with rats,[24,25,39,58,59] quail,[60] and squirrel monkeys.[61] It ensures that accelerations are applied to the semicircular canals each time rotation begins and ends. The occurrence of accelerations can also be ensured easily by tilting the rotation platform so the axis of rotation deviates from earth vertical. When the platform is so tilted, the body axis of rats is oscillated between head up and head down positions during rotation, thereby applying a sinusoidal pattern of accelerations to the otoliths. This method has been used with rats[57] and mice.[38]

Other methods of ensuring the application of accelerative forces to the vestibular system have

involved more-complicated motion devices. The effects of centrifugation have been investigated using forces of 5 to 10 times gravity.[62] Rotation about two axes simultaneously was accomplished using a modified Hobart mixer[33] with the extreme rotary speeds of 198 rpm and 88 rpm combined. Rotation combined with sinusoidal vertical oscillation has been used to produce CTA in squirrel monkeys,[34] but vertical sinusoidal acceleration alone failed to produce CTA in squirrel monkeys.[63] Simple, vertical axis rotation can be used to produce CTA in squirrel monkeys,[35,64] so it appears that rotation may have been the effective stimulus in the earlier study[34] when rotation was combined with a vertical excursion of the apparatus.

Several experiments have demonstrated that the magnitude of motion-induced CTA is affected in a predictable manner by manipulation of parameters of motion which are known to affect the severity of motion sickness in man. Several variables have been manipulated to provide such correlational evidence. Green and Rachlin[15] investigated the magnitude of rotation-induced CTA while varying both the duration of exposure to rotation and the speed of rotation. Their analysis indicated that the absolute number of rotations, not the speed or duration of rotation alone, was the best predictor of the magnitude of aversion. The effect of different forms of passive motion on the magnitude of CTA has been investigated for three different motion profiles.[65] Accelerative forces were varied by using three conditions producing increasing stimulation to the vestibular apparatus. As the degree of presumed vestibular stimulation increased from a condition involving only vertical-axis rotation, to sinusoidal bouncing (seesaw motion), to cross-coupled motion comprised of rotation during seesaw oscillation, the magnitude of CTA also increased. Off-vertical rotation has also been used to address this issue. Off-vertical rotation becomes increasingly provocative for producing motion sickness as the degree of tilt increases and approaches "barbeque spit rotation".[66] Fox et al.[38] demonstrated that the magnitude of CTA increased as the tilt-axis of a rotation platform was increasingly deviated from earth vertical.

B. METHODS OF CAGING DURING EXPOSURE TO MOTION

1. Individual vs. Group Caging

Considerable improvement in methodological efficiency can be accomplished by exposing animals to rotation in groups rather than individually. The amount of savings obtained by this procedure obviously increases as the duration of the rotation period is lengthened or as the number of animals is increased. Because the magnitude of CTA can be influenced by circadian rhythm,[67] conditioning should be conducted during a limited period of the day. This can be facilitated by using several motion devices, by distributing the experiment over several days, or by exposing several animals to motion simultaneously.

These issues are addressed briefly by Harrison and Elkins,[68] who indicated several previous studies using various approaches to expose small groups of rats to rotation. They also describe a simple, easily constructed device for exposing groups of rats to rotation. Their device confines rats in tubes constructed of PVC pipe. Two tubes are placed side by side, and two tiers are stacked so that four rats can be rotated simultaneously. A similar tiered approach has been used with four compartments (18 × 19 × 10 cm) in each of five tiers, permitting the simultaneous exposure of up to 20 rats to off-vertical rotation.[69,70] Placement of animals side by side with the axis of rotation between them permits two animals to be close to the axis of rotation on each level. Placement of animals in chambers constructed as small squares within a larger square pattern with one corner of each of the smaller squares converging over the axis of rotation permits four animals to make voluntary movements close to the axis of rotation on each level of such a device. These approaches facilitate the testing of several animals while confining all animals close to the axis of rotation and thereby minimizing centrifugal forces which increase with increasing displacement from the axis. The total number of animals that can be exposed at a time can then be increased by stacking levels up to the safety limits of the rotation device. The expansion of such devices for use with larger animals should be done with consideration of possible safety

factors resulting from weight of the device and the animals. In addition, as larger confinement chambers are used with larger animals, greater centrifugal forces can result from orientations adopted by the animals. Thus, control of the effective stimulus serving as the US depends increasingly on the orientation adopted by the animal during rotation. Larger animals such as monkeys or cats are typically exposed to motion individually. A device for exposing two cats to motion simultaneously has been described,[71] but this device has not been used in studies of CTA.

2. Restriction of Voluntary Movement

As reflected by vomiting, motion is dramatically less provocative in man when head movements are restricted[72] and in squirrel monkeys when movement is restricted,[73] or prevented by rigid restraint.[61,74,75] From the viewpoint of experimental control, however, the restriction of movement during exposure to motion has the beneficial effect of permitting better specification of accelerations to the vestibular system. This benefit derives from the elimination or reduction of accelerations dependent on movement by the animals. Restriction of movement thus tends, in effect, to equate stimulation which otherwise might vary due to movements elicited or evoked differentially in individual animals exposed to motion.

Restraint has not been used often in studies of motion-induced CTA. The movement of rats has been restricted to avoid uncontrolled, cross-coupled accelerations produced by whole-body movement when investigating CTA induced by centrifugation.[62] In addition, restraint has been used when exposing rats to off-vertical rotation.[76]

The magnitude of CTA induced by off-vertical rotation with whole-body movement of rats permitted or restricted was investigated in an unpublished experiment. Rats in a voluntary movement condition (FREE) were placed in opaque plastic mouse cages (8 × 18 × 28 cm) when exposed to rotation. Rats in the restricted movement condition (RESTRAINED) were placed in plastic tubes 8 cm in diameter and 18 cm long during exposure to rotation. Each of 32 rats was assigned randomly to one of the eight conditions formed by the factorial combination of two treatment conditions (motion or no motion), two novel flavors (sucrose or salt), and two rotation conditions (free or restrained). The rotation profile consisted of off-vertical rotation (rotation axis displaced 20° from earth-vertical) and an angular velocity of 150°/s for 15 min. A discrimination procedure adapted from that used by Braun and McIntosh[37] was used for the conditioning procedure. During an 8-d acclimation period, rats were adjusted to a restricted drinking procedure consisting of 15 min of access to tap water in the home cage every 12 h followed immediately by placement in the appropriate experimental holding cage for 15 minutes. During conditioning, one of two taste solutions, either sucrose or salt, or tap water was offered in each drinking session (i.e., one every 12 h). One taste solution was always followed by exposure to rotation. Tap water was offered in the drinking session 12 h after rotation and the other taste solution was offered in the drinking session 24 hours after rotation. Completion of three consecutive drinking sessions, during which each of the three fluids was offered once for drinking, comprised a conditioning cycle of the procedure. Six conditioning cycles were used in the experiment.

Conditioning was much stronger to the salt taste than to the sucrose taste. The median intake of the paired taste solutions by animals in the FREE and RESTRICTED movement conditions is shown in Figure 1. Each curve is based on data from only four animals and consequently should be interpreted with caution, but there is no evidence in these data of any reduction in the magnitude of CTA when whole-body movement is restricted. Neither parametric nor nonparametric statistical tests indicated a reliable difference between the conditions ($ps > 0.20$). Thus, although the assessment of motion sickness by vomiting indicates reduced sickness under conditions of restricted movement, the magnitude of motion-induced CTA was not reduced when movement was restricted during exposure to off-vertical rotation.

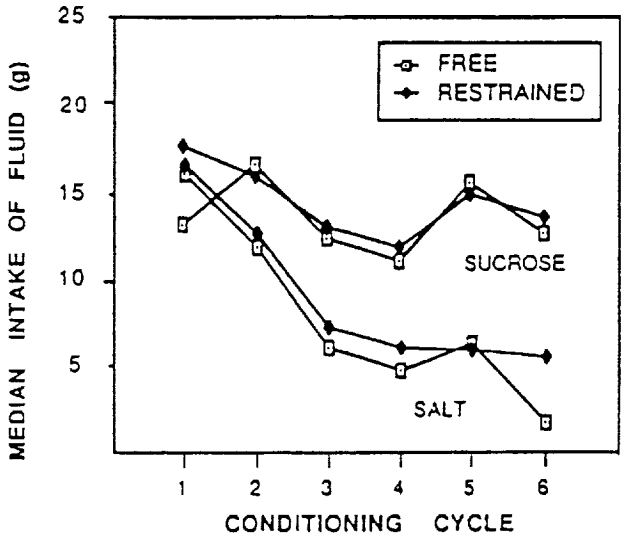

FIGURE 1. Median intake of the solution which was paired with motion when animals were permitted to make voluntary movements (FREE condition) or when movement was restricted (RESTRAINED condition) during motion. The upper curves reflect conditioning when the sucrose solution was paired with motion. The lower curves reflect conditioning when the salt solution was paired with motion. Each curve is based on data from four rats.

C. TYPES OF CONDITIONED STIMULI AND METHODS OF PRESENTATION

Flavored fluids have been used as CSs most commonly, but solid foods have been used with rats[65] and squirrel monkeys.[35,74] Fluids generally are preferred over solid foods as CSs because residual traces are not as likely to be present after the CS is removed at the end of the period of access. Solid foods may remain on the fur of the animal and be encountered during grooming after exposure to motion. When the CS is presented in the home cage of the animal, spilled or smeared food may remain and be eaten after the animal is returned following treatment with motion. Nonnutritive substances are generally preferred over nutritive substances to avoid confounding nutritional consequences with the effects of illness.

Most studies which have used flavored fluid as the CS have assessed the magnitude of CTA with the "two bottle" method. With this method, the CS and tap water are available simultaneously during tests for conditioned aversion. The magnitude of CTA is assessed as preference for the flavored fluid determined as the percentage of total fluid intake accounted for by intake of the CS. With the "one bottle" method, only one fluid is offered for drinking in a single period of restricted access each day. With this method, aversion to the CS is shown either as lesser consumption of the CS after exposure to motion than before that exposure (a within-subjects comparison) or as lesser consumption of the CS by animals exposed to motion than by control animals not exposed to motion (a between-subjects comparison). The two-bottle method is generally considered to be a more sensitive test of CTA than is the one-bottle method.[77,78] However, Ossenkopp[59] found that enhancement of motion-induced CTA in animals with the area postrema lesioned was detected with an intake measure (one-bottle method) but not with a preference measure. He concluded that the preference measure was not sensitive to this enhancement effect in his experiment because preference for the CS was so low that it could not be reduced (i.e., a "floor effect" prevented detection of the enhancement of CTA). Thus, under some conditions the one-bottle method might be preferred.

The discrimination procedure used by Braun and McIntosh[57] and in the unpublished

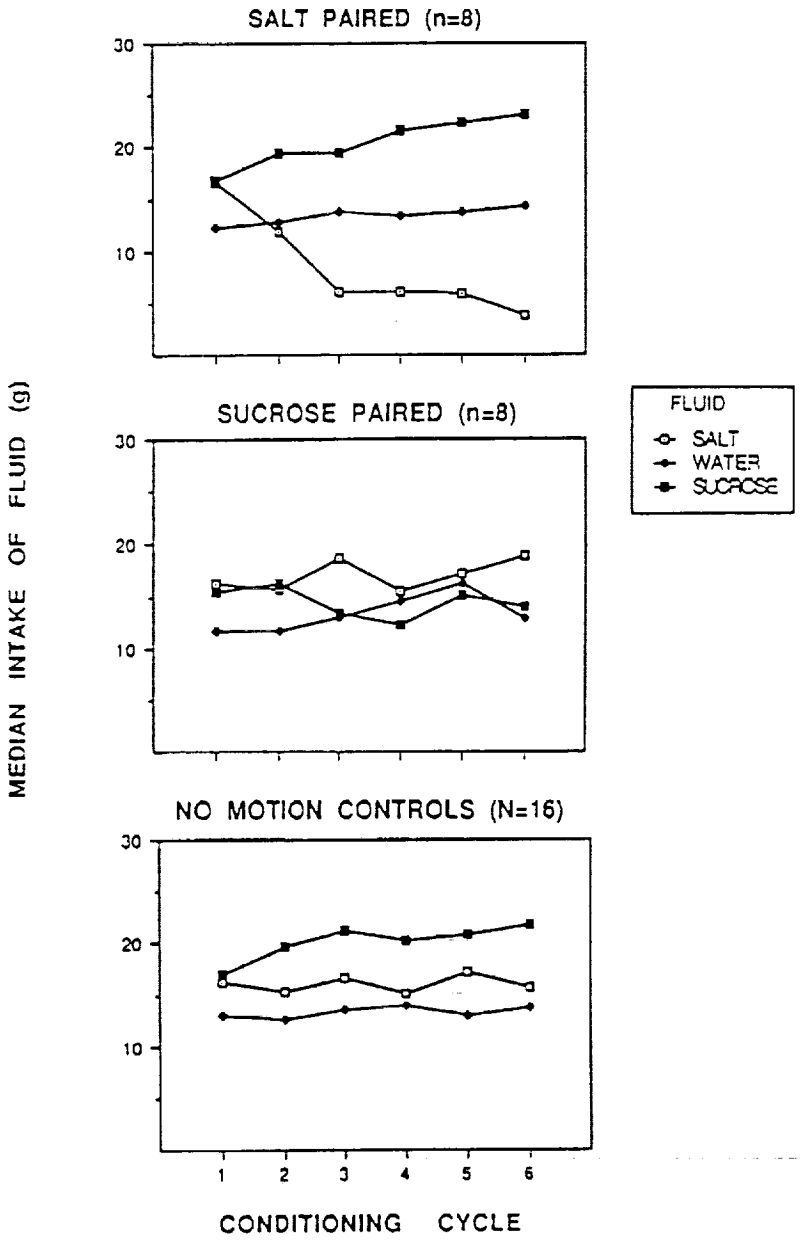

FIGURE 2. Median intake of the three solutions consumed during the experiment. Conditioning effects are shown in the upper panel where the salt solution was paired with motion and in the middle panel where the sucrose solution was paired with motion. Median intake reflecting preferences for the solutions by animals which were never exposed to motion are shown in the bottom panel.

experiment discussed above may be prone to spurious effects arising from repeated exposure to more than one flavored stimulus. Additional data from that unpublished experiment are presented in Figure 2 and show the intake of each fluid with the FREE and RESTRAINED conditions combined. It is apparent from this figure that a stronger aversion was produced when salt was paired with motion (upper panel) than when sucrose was paired with motion (middle panel). When the salt solution was paired with motion, the animals consumed less of that solution

in all tests for aversion (Cycles 2 through 6, $p < 0.01$). However, when the sucrose solution was paired with motion, the consumption of that solution was reduced only in Cycles 3 and 4 ($p < 0.05$). Intake data for the control animals not exposed to motion are shown in the bottom panel of the figure. For these animals both flavored solutions were preferred over tap water in Cycle 1 ($p < 0.01$), but neither flavored solution was preferred over the other ($p > 0.05$). However, after repeated exposure to the three fluids, consumption of the sucrose solution increased and this solution came to be preferred over the salt solution ($p < 0.01$ in Cycle 6) while consumption of the salt solution and tap water did not vary ($p > 0.05$). When these results are combined, it can be seen that a weak aversion developed to the paired solution which became more preferred during the experiment (sucrose), while a strong aversion developed to the paired solution for which preference remained stable during the experiment. This observation indicates that the magnitude of motion-induced aversions may be related to the preference for flavored cues used as CSs.

It should be noted, however, that these results differ from those reported by Braun and McIntosh in two ways: they found aversions of similar magnitude to both flavored solutions and, although the consumption of the sucrose solution was greater than that of the salt solution in their experiment, they did not report a statistically reliable difference in consumption. There is insufficient information available in the Braun and McIntosh paper to perform a post hoc analysis to evaluate more completely the possibility of a shift in preference in their control group. The issue of the strength of aversion to sucrose may be related to the fact that a more severe stimulus was used by Braun and McIntosh. In that experiment, rats were exposed to off-vertical rotation at 150 rpm for 5 min (750 total revolutions) while in this experiment they were exposed to off-vertical rotation at 25 rpm for 15 min (325 revolutions). Thus, if the total number of revolutions is used to evaluate the intensity of the US,[15] there is an intensity ratio of 2:1 in the two experiments. These observations indicate that the effects of preference on the magnitude of motion-induced CTA may become evident only if moderate motion profiles are used. In addition, it appears that there may be complicated interactions between the preferences for solutions used as CSs, the number of exposures to flavored cues and changes in preferences for them, and the intensity of motion which is used as an US.

V. RELATIONSHIP OF CTA TO NAUSEA AND VOMITING

A presumed relationship between gastrointestinal disturbance and the development of CTA is particularly important to the use of CTA for the study of motion sickness. The use of CTA as a putative measure of motion sickness in species which do not vomit (i.e., rats) rests on the assumption that motion-induced CTA is produced via neural and physiological states which either are the same as or are analogous to those which produce vomiting in species with a complete emetic reflex. When CTA is used as an index of subemetic levels of motion sickness[34] or "concomitant" symptoms of motion sickness,[93] it is assumed to reflect states which comprise internal sequelae progressing toward vomiting or internal states comprising the motion sickness syndrome. A relationship between CTA and visceral disturbance in the form of either nausea or vomiting has been implicit in various reports. Wilpizeski and Lowry[35] provide a formal theory of motion sickness in squirrel monkeys in which they propose that CTA reflects the development of a "nausea factor" which is independent of an "emetic factor" that underlies vomiting.

A. CTA PRODUCED BY DRUGS

However, the validity of this assumed relationship between gastrointestinal disturbance and the development of CTA induced in animals by drug treatments remains open to criticism. Ashe and Nachman[16] pointed out that the efficacy of several drugs and of irradiation as USs is not correlated strongly with the effectiveness of those treatments in producing gastric dysfunction. Dose levels of several drugs and irradiation which are too low to produce obvious signs of

sickness in animals can be very effective treatments for producing CTA, and doses of apomorphine which produce indications of extreme sickness may produce a CTA of relatively low magnitude.

Thus, it appears to be more accurate to consider gastric illness to be a sufficient, but not a necessary condition for the production of drug-induced CTA, than to assert that gastric illness is the functional stimulus serving as an US in drug-induced CTA.

Information about the relationship between nausea and CTA induced by toxins can be obtained from reports of nausea in patients studied for the development of CTA while undergoing cancer therapy. Experimental control is very difficult in clinical studies, but it appears from such studies that CTA and nausea are not inextricably interdependent. It has been reported that the likelihood of developing CTA during radiation therapy is related to the site of application of irradiation and that CTA does not always occur when nausea is reported.[2] Conversely, CTA may occur when nausea is not reported. Bernstein and Webster[79] also reported the development of CTA in patients not reporting nausea. The degree of nausea reported by their patients was unrelated to the magnitude of aversion. Thus, the predictability between nausea and CTA appears to be poor, but the reasons for this are unknown. More objective assessment of the degree of nausea in patients might improve predictability. The level of plasma vasopressin has been shown to be related to nausea in humans,[80] and might be used for more objective assessment. However, this technique would require an invasive procedure with patients. While there is evidence that plasma vasopressin is related to the emetic reflex in cats,[81] a convincing demonstration that increased levels of plasma vasopressin reflect nausea in animals remains to be provided, and this technique has not been used in investigations of CTA.

B. CTA PRODUCED BY MOTION

Investigations of motion-induced CTA in animals with a complete emetic reflex provide evidence indicating CTA and vomiting are not directly related. In studies with cats[82] and squirrel monkeys,[34,35] CTA has been reported in animals which did not vomit in response to motion. In addition, not all animals which did vomit developed CTA. Thus, if CTA was produced by visceral illness, vomiting is not a completely reliable index of that illness. That vomiting is not the sole index of motion sickness is acknowledged, of course, when rating scales based on putative prodromal symptoms are used with humans[83] or animals.[84,85]

C. RELATED RESEARCH

Research investigating the effects of antiemetic drugs on CTA in rats provides additional related information on the relationship between nausea and CTA. These studies have used the rationale that if nausea plays an important role in CTA, it might be possible to use antiemetics to prevent either the formation or expression of CTA. One approach might be to prevent CTA by the administration of an antiemetic before exposing the animal to the US and inducing nausea. This procedure is questionable because some antiemetics (i.e., scopolamine) can serve as USs to produce CTA. Thus, an antiemetic administered to counteract a presumed nauseogenic effect of an US might enhance the magnitude of CTA. The antiemetic dose of a drug typically is considerably less than the dose that serves as an US for producing CTA, but the potential for confounding is clearly present in such a procedure. Consequently, most studies have investigated whether antiemetics administered at the time of testing for CTA reduce the magnitude of that CTA. If the magnitude of CTA is attenuated in animals treated with an antiemetic prior to testing, it might be argued that the antiemetic counteracted conditioned nausea elicited by the taste cues (CS) at testing.

Studies which have investigated whether antiemetics administered prior to testing do attenuate CTA have produced inconsistent results. When CTA was induced by lithium chloride injection, the administration of scopolamine, cyclizine, prochlorperazine, or trimethobenzamide before testing was reported to attenuate the magnitude of CTA.[86] However, a later study

failed to replicate this finding.[87] In this second study, no attenuation of CTA was found when prochlorperazine or scopolamine was administered prior to testing for CTA induced by the injection of lithium, amphetamine, or morphine as USs. Replication failed even though strong as well as weak aversions were produced and a range of antiemetic doses was used. This outcome is in agreement with an earlier report of no attenuation of CTA when scopolamine was administered prior to testing for the CTA.[88]

Studies conducted to investigate the role of selected neural structures in CTA induced using motion as the US also provide some information related to the relationship between CTA and vomiting (see work by Fox et al.[89] for a more detailed discussion than is provided here). When exposed to passive motion after complete ablation of the area postrema, rats develop CTA,[59,69] and cats[42] and squirrel monkeys[64] develop CTA and vomit. Thus, the area postrema apparently does not play an essential chemoreceptive role in either CTA or vomiting induced by motion. After selective gastric vagotomy, CTA was not produced in rats when vertical axis rotation was the US.[70] Whether vagal pathways might be shared by CTA and vomiting could not be addressed directly in this experiment because rats are incapable of vomiting, but it seems unlikely that the crucial neural pathways for these two responses are isomorphic because vagotomy does not eliminate motion-induced vomiting in dogs.[90]

These studies of neural structures have not elucidated a single neural mechanism that mediates motion-induced CTA. However, it has been shown that both vagal and vestibular functions[25,26] contribute essentially to the production of CTA in rats when motion is the US. Perhaps motion-induced CTA depends on the convergence of vagal and vestibular functions, or on some unknown neural network which receives inputs from various structures (i.e., vagus nerve, vestibular system, area postrema, etc.). Also, it is known that the rate of gastric emptying is affected by vestibular stimulation,[91] that afferent activity in the vagus nerve is influenced by caloric stimulation,[92] and that tachygastria is associated with prodromal symptoms of motion sickness.[93] Whether the neural structures essential to the development of CTA induced by motion also are essential to vomiting induced by motion is unknown at this time.

VI. SUMMARY AND CONCLUSIONS

CTA was proposed as a measure of motion sickness due, in part, to the commonly accepted concept that visceral sickness is the functional US for drug-induced CTA. In early studies of CTA induced by drugs, it was shown that this presumed visceral illness is associated uniquely with gustatory cues rather than with exteroceptive cues. Several studies have shown that taste aversion is not formed to exteroceptive stimulation present at the time of exposure to motion. Thus, gustatory cues are assumed to be associated uniquely with aversive, interoceptive effects of motion rather than with any of the various exteroceptive effects associated with exposure to motion.

The use of CTA to measure motion sickness also is supported by studies showing that an intact vestibular system is essential for the production of CTA when motion is the US. This finding parallels the well known absence of motion sickness in humans and animals with defective or damaged labyrinths. In addition, the magnitude of CTA is increased by longer exposure to motion and by manipulations which increase vestibular stimulation (i.e., by off-vertical rotation). Thus, certain changes in the parameters of motion that affect the production of motion-induced vomiting also affect the presence or magnitude of motion-induced CTA.

CTA has two principle advantages over some of the other putative measures of motion sickness. The magnitude of CTA is assessed at a time removed from exposure to motion, and therefore is not affected by residual effects of motion (i.e., by disorientation, disruption of locomotion, etc.). Some of the other indices may be affected by these factors and therefore can lead to false positive indications of motion sickness. Second, because the magnitude of CTA is assessed as volume or weight of food or fluid, the degree of sickness is reflected in a continuous

measure rather than in the discrete, all-or-none fashion characteristic of vomiting. A possible third advantage might be that CTA provides a very sensitive measure of motion sickness. The use of CTA to measure subemetic levels of motion sickness is based upon this concept. However, it should be recognized that this application assumes CTA is not only more sensitive than vomiting, but also that CTA reflects prodromal symptoms progressing toward vomiting as the endpoint of motion sickness.

As with other measures, there can be complicating factors and potential disadvantages involved when CTA is used to assess motion sickness. Conditioned aversion is a *learned response*, and therefore is qualitatively different from the universally accepted index of motion sickness, the emetic *reflex*. Because CTA is a learned response, various control conditions commonly used in the study of learning mechanisms may be required in specific applications of the method. Control conditions for assessing psuedoconditioning and various other artifactual effects may require significant additional expense and work in an experiment. The importance of these control conditions is less critical if CTA is used as a discrete assessment of motion sickness (present or absent). However, if an experiment requires precise comparison of the magnitude of CTA produced by different treatments, control conditions become paramount. In addition, repeated testing of animals as conducted in within subjects designs may be contraindicated by the potential for the magnitude of CTA to be affected by both variation in the novelty of the CS and exposure to motion or drugs prior to conditioning.

There are three areas where assessments of motion sickness using CTA and other measures appear to differ. First, neither nausea nor vomiting seem to have an essential, direct relationship to motion-induced CTA. This reflects negatively on the use of CTA to assess motion sickness because both vomiting and nausea are principle indices of motion sickness. Second, the restriction of movement during exposure to motion may not reduce the magnitude of CTA produced by that motion. This is in contradistinction to the reduction in vomiting that occurs when the movement of humans and animals is restricted. This point should be considered cautiously, however, because it is based on a single, preliminary test: conclusive resolution of this issue requires more extensive experiments. Third, it appears that CTA and motion sickness might depend upon different neural structures. The meaning of recent evidence indicating that motion-induced CTA is prevented in rats by selective gastric vagotomy is unclear at this time. Previous research has led to the general conception that abdominal innervation plays no essential role in motion-induced vomiting. This apparent difference in neural mechanisms may arise from differences between the nervous systems of rats and species possessing a complete emetic reflex. Alternatively, a demonstration that motion-induced CTA is prevented by selective gastric vagotomy in species possessing a complete emetic reflex might imply that the abdominal vagal system is involved in some manner, if not essentially, in motion sickness. Although the area postrema was long thought to be essential for the production of vomiting by motion, we now know that both CTA and vomiting can be produced by motion after the area postrema has been completely ablated. Perhaps additional research will elucidate neural systems common to, and different between, motion sickness and CTA.

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Conditioned taste aversion and motion sickness in cats and squirrel monkeys[1,2]

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The relationship between vomiting and conditioned taste aversion was studied in intact cats and squirrel monkeys and in cats and squirrel monkeys in which the area postrema was ablated by thermal cautery. In cats conditioned 7–12 months after ablation of the area postrema, three successive treatments with xylazine failed to produce either vomiting or conditioned taste aversion to a novel fluid. Intact cats, however, vomited and formed a conditioned aversion. In squirrel monkeys conditioned 6 months after ablation of the area postrema, three treatments with lithium chloride failed to produce conditioned taste aversion. Intact monkeys did condition with these treatments. Neither intact nor ablated monkeys vomited or evidenced other signs of illness when injected with lithium chloride. When the same ablated cats and monkeys were exposed to a form of motion that produced vomiting prior to surgery, conditioned taste aversion was produced and some animals vomited. These findings confirm other studies indicating motion can produce vomiting in animals with the area postrema destroyed and demonstrate that motion-induced conditioned taste aversion can be produced after ablation of the area postrema. The utility of conditioned taste aversion as a measure of subemetic motion sickness is discussed by examining agreement and disagreement between identifications of motion sickness by conditioned taste aversion and vomiting. It is suggested that a convincing demonstration of the utility of conditioned taste aversion as a measure of nausea requires the identification of physiological correlates of nausea, and caution should be exercised when attempting to interpret conditioned taste aversion as a measure of nausea.

Key words: area postrema, conditioned taste aversion, motion sickness, nausea, emesis.

FOX, R. A., CORCORAN, M., et BRIZZEE, K. R. 1990. Conditioned taste aversion and motion sickness in cats and squirrel monkeys. Can. J. Physiol. Pharmacol. **68** : 269–278.

On a étudié la relation entre le vomissement et l'aversion gustative conditionnée chez des chats et des singes écureuils intacts et chez des chats et des singes écureuils dont l'area postrema avait été détruite par thermocautérisation. Chez les chats conditionnées 7–12 mois après l'ablation de l'area postrema, trois traitements successifs à la xylanine n'ont pu provoquer de vomissement ni d'aversion conditionnée à un nouveau liquide. Les chats intacts, toutefois, ont eu des vomissements et ont développé une aversion gustative conditionnée. Chez les singes écureuils conditionnés 6 mois après l'ablation de l'area postrema, trois traitements au chlorure de lithium n'ont pu provoquer d'aversion conditionnée. Les singes intacts ont développé un conditionnement avec ces traitements. Ni les singes ayant subi une ablation ni les singes intacts n'ont eu de vomissement ou montré d'autres signes de maladie après avoir reçu une injection de chlorure de lithium. Lorsque les chats et singes ayant subi une ablation ont été exposés, avant l'opération, à une forme de mouvement provoquant le vomissement, une aversion gustative conditionnée a été observée et certains animaux ont eu des vomissements. Ces résultats confirment d'autres études indiquant que le mouvement peut provoquer des vomissements chez les animaux dont l'area postrema a été détruite, et démontrent que l'aversion gustative conditionnée induite par le mouvement peut être produite après l'ablation de l'area postrema. On discute de l'utilité de l'aversion gustative conditionnée en tant que mesure de mal des transports sous-émétique, en examinant les points communs et divergents en ce qui a trait à l'identification du mal des transports par le biais de l'aversion gustative conditionnée et des vomissements. On suggère qu'une solide démonstration de l'utilité de l'aversion gustative conditionnée en tant que mesure de la nausée requiert l'identification de corrélats physiologiques de la nausée. L'aversion gustative conditionnée en tant que mesure de nausée doit être interprétée sous réserve.

[Traduit par la revue]

Introduction

The conditioned taste aversion (CTA) procedure was introduced and studied extensively by Garcia (1981) and his colleagues as a unique example of classical conditioning in animals. Although the various toxic treatments used to produce CTA are referred to as unconditioned stimuli (USs), Garcia and Ervin (1968) proposed that malaise or disruption of the "mileau interne" was the stimulus that effectively served as the US to produce conditioning. Thus, they proposed that the internal consequences of toxic treatments ("illness") are associated with recently ingested novel food substances (i.e., with foods ingested contiguously with those internal consequences). In this scheme, the obvious biological utility of CTA is that it protects animals from repeated exposure to toxic foods that threaten survival.

Following Garcia's argument and various forms of empiri-

[1]This paper was presented at the symposium Nausea and Vomiting: A Multidisciplinary Perspective, held November 12 and 13, 1988, Ottawa, Ont., Canada, and has undergone the Journal's usual peer review.

[2]Portions of these data were reported at the meeting of the American Physiological Society (Experiment 2: Corcoran et al. 1985, Physiologist, **28**: 330) and the Society for Neuroscience (Experiment 1: Elfar et al. 1986, Neurosci. Abstr. **12**: 885).

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cal evidence, there has been a persistent acceptance of the concept that illness serves as the proximal US producing CTA. Many nauseogenic drugs and procedures that produce internal malaise also produce CTA. For example, food aversions may be formed in humans during the course of medical regimens, as in chemotherapy (Bernstein 1985) or alcohol aversion therapy (Logue 1985), further supporting the concept that illness serves as the US in the production of CTA.

On the other hand, illness and CTA are not related in a predictable manner in all instances. Treatment with some drugs (e.g., amphetamine, scopolamine) can produce CTA even though the drugs are administered at doses that produce no other signs of sickness (see Ashe and Nachman, 1980, and Gamzu et al., 1985, for reviews of this issue). In addition, certain agents known to be toxic, and known to produce illness, apparently cannot be used to produce CTA (Riley and Tuck 1985). Reconciliation of these inconsistencies between illness and CTA is difficult because the physiological and neural events that underlie illness (e.g., the nausea–emesis syndrome) have not been precisely specified. Furthermore, objective measures of illness are limited. Emesis is generally identified objectively, although exact identification by observation may be difficult in animals (and not all animals have a complete emetic reflex). Nausea, however, typically is identified by self-report, and there are few techniques available for directly assessing its presence or degree. Plasma vasopressin is elevated (Rowe et al. 1979) and tachygastria has been reported during nausea in man (Stern et al. 1985), but these measures are not well documented for animals. Plasma vasopressin is elevated for 3–6 min after vomiting in cats, but its level before vomiting when nausea is expected has not been identified because the precise time of vomiting cannot be anticipated (Fox et al. 1987). We are unaware of documentation of tachygastria associated with vomiting in animals.

Using the rationale that the neural pathways important to nausea/vomiting should mediate CTA if illness is the proximal US for conditioning, two recent articles have investigated the neural mechanisms important to motion-induced CTA in rats. Because the area postrema (AP) is crucial to CTA induced by blood-borne toxins such as lithium chloride (LiCl) (Ritter et al. 1980) and intravenous copper sulfate (Coil and Norgren 1981) in rats, and the AP also serves as a chemoreceptive site of action for emetic effects of several drugs, including xylazine in cats (Borison et al. 1984) and X-irradiation in dogs (Wang et al. 1958) and the squirrel monkey (Brizzee et al. 1955), the possible role of the AP in motion-induced CTA in rats has been investigated. Ossenkopp (1983) reported that motion-induced CTA was enhanced in rats with the AP ablated. Sutton et al. (1988) did not find direct enhancement of CTA in AP-ablated rats but did report slower extinction of motion-induced CTA in rats with the AP ablated. In both studies, destruction of the chemoreceptive function of AP was demonstrated by the ineffectuality of drugs (scopolamine methyl nitrate by Ossenkopp, 1983, and LiCl by Sutton et al., 1988) for producing CTA in these same ablated rats. Thus, both of these studies show that motion-induced CTA in the rat can occur when the AP is destroyed. No objective measures of illness were reported in either study, however, so any possible relationship between illness and CTA could not be assessed directly in these experiments.

Because the relationship between illness and CTA was crucial to our interest in using the CTA paradigm to study motion sickness, we conducted similar experiments using the cat and squirrel monkey so that vomiting could provide an objective measure of illness. By studying CTA in species with a complete emetic reflex, we hoped to make a more direct investigation of the relationship between illness and CTA. In addition, we ablated the AP in both species to investigate further the role of this structure in motion-induced CTA and vomiting.

General methods

Subjects

Twenty-three adult male squirrel monkeys and 26 adult female cats were selected from the pool of animals used in motion sickness research. All animals were housed either in individual cages or in runs at the Ames Research Center Animal Care Facility on a 14-h light – 10-h dark cycle (monkey) or an 8.5-h light – 15.5-h dark cycle (cat). For 4 h (monkey) or 22 h (cat) prior to each conditioning session, the animals were deprived of food and water.

Surgical procedures

Bilateral ablation of the AP was carried out in 7 adult monkeys and 10 adult cats. Aseptic precautions were employed in all surgical procedures. The trachea was intubated with a plastic tracheal tube (2.5 mm for monkeys and 3.5 mm for cats) and pulmonary ventilation was supported artificially. Halothane inhalation anesthesia was used. The neck was extended and strongly ventroflexed by the use of a head holder to give good access to the foramen magnum. The occipital bone and first cervical vertebra were exposed by lateral retraction of the nuchal muscles. An opening was made in the lower portion of the occipital bone to expose the cerebellar area and lower medulla. A midline sagittal incision was made in the dura mater and cerebellomedullary pia-arachnoid. The cerebellum was then gently displaced upward by means of a small paraffin-coated spatula. The medullary velum was also incised and the floor of the fourth ventricle was exposed to direct vision.

During the AP ablation operation, the operative field was kept dry and free of CSF by continuous aspiration rostral to the operative area. The AP was ablated by free-hand thermocoagulation with the aid of an operating microscope at 6× magnification. The cautery tip, a 42-gauge stainless steel wire loop inserted into a pen-type handle, was energized with three AAA batteries operated by a foot switch to a level below red heat. Because three different neurosurgeons indicated during consultation that the dura heals and closes a surgical opening very rapidly without being sutured, the dura was not sutured following the ablation. Rather, the edges of the sagittally incised pia-arachnoid and dura mater were brought into approximation and the neck muscles were sutured together over the dura, thus holding it in place. The skin was then closed by interrupted sutures with 3-0 silk.

During recovery, animals were treated during the 1st week with analgesics as deemed necessary by the attending veterinarian. For variable periods after surgery, animals showed a sharp decrease in voluntary movement, and initially both cats and monkeys typically refused food. Normal feeding and activity returned after 10–15 days. After recovery, the lesioned animals could not be distinguished from intact animals.

Conditioning was conducted 6 months (monkeys) and 7–12 months (cats) following surgery. These lesioned animals were used in other studies of motion sickness before and after the CTA experiments were conducted.

Histological procedure

Following completion of this and the other experiments, the animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with saline, followed by a solution of Formalin (and acetic acid and methanol for monkeys). Blocks of tissue were stored in Formalin for 2–3 weeks before being prepared for light microscopy. Brainstems were embedded in paraffin and 10-μm serial coronal sections were cut at the level of the AP. Sections were mounted on microscope slides, stained with hematoxylin and eosin, and evaluated for completeness of AP ablations and for any damage to adjacent structures. Monkeys were perfused 1 year after ablation

of the AP, and cats were perfused 8–12 months after ablation of the AP.

Conditioning procedures

Conditioned taste aversion was studied using a "one-bottle" conditioning paradigm. A 30-min drinking period in which animals had access to a novel fluid (the CS) was immediately followed by the experimental treatment (injection of a drug or exposure to motion) or a control treatment (injection of saline). Conditioning sessions occurred every 3 days.

Conditioning was accomplished in two phases, with different treatments used as the US in the first and second phase. All animals had three conditioning sessions in phase I (days 1, 4, and 7). On day 10, animals being conditioned only in phase I (i.e., conditioned with only one US) had the 30-min drinking period only, while animals being conditioned in both phases were exposed to the second treatment immediately after the drinking period on day 10. For those monkeys conditioned in phase II, two more conditioning sessions occurred (on days 13 and 16) followed by an additional drinking session on a final day (day 19) to assess the effects of the sixth conditioning session. For those cats conditioned in phase II, one more conditioning session occurred (on day 13) followed by a final drinking session (day 16).

Animals were observed continuously for 1 h after injections to determine whether vomiting occurred. In the event vomiting had not occurred within the 1st h after injection, periodic checks of the cage were conducted at intervals of approximately 10 min for evidence of vomitus. Similarly, periodic checks of the cage of each animal were conducted at 10-min intervals for 1 h after motion was terminated. The animals typically appeared relaxed and normal as evidenced by voluntary locomotion by the end of this observation period.

Experiment 1: Monkeys

Method

Yellow, sweet, almond-flavored water (50 g sucrose, 0.2 mL food color, and 1.5 mL almond flavor in 1.0 L of water) was used as the CS. During each conditioning session, the CS was available in standard drinking bottles mounted on the side of a ventilated, clear Plexiglas cage of the same size as the cage used for rotation as described below. Animals were transferred to these cages 10 min before the beginning of the 30-min drinking period to permit acclimation to the test room. The amount of fluid consumed in each drinking period was determined by weighing drinking bottles, and the latencies of retches and vomits during treatments were recorded. Monkeys were observed for 30 min after treatments to determine whether vomiting occurred. No animal vomited during this observation period, and no evidence of vomiting was detected in periodic checks over the following hour.

Monkeys were assigned to four groups defined by the treatments used as USs in conditioning sessions as follows.

Group 1: Conditioned with motion in phase I only ($n = 5$). These animals were individually exposed to counterclockwise rotation about the vertical axis for 30 min at 150°/s in a ventilated, clear Plexiglas cage (52 × 23 × 30 cm) within 5 min after removal of the CS at the end of the drinking period.

Group 2: Conditioned with LiCl in phase I only ($n = 6$). These animals were injected intraperitoneally with 0.3 M LiCl (5 mL/kg) within 5 min after removal of the CS.

Group 3: Conditioned with saline in phase I followed by motion in phase II ($n = 5$). In phase I, animals were injected intraperitoneally with 0.9% NaCl (5 mL/kg) within 5 min after removal of the CS. In phase II (beginning on day 10), the animals were exposed to rotation as described for group 1.

Group 4: AP-lesioned animals conditioned with LiCl in phase I followed by motion in phase II ($n = 7$). In phase I, animals were injected with LiCl as described for group 2 above. In phase II, these animals were exposed to rotation (as described for group 3).

Results

Histology of AP ablations

Sections through the caudal medulla of the AP of an unoperated control (right column) and a lesioned monkey (left column) are shown in Fig. 1 to illustrate the general extent of the lesions. The AP was destroyed in all animals and limited damage occurred to peripostremal structures with varying amounts of damage occurring in different animals. The tractus solitarius was intact in all animals.

Phase I conditioning

All of the rotated monkeys vomited during each of the conditioning sessions, but no monkey vomited after injection with LiCl or NaCl. For the rotated monkeys, the latencies to vomiting ranged from 1 to 8 min. The average consumption of the CS for the four groups of monkeys is shown in Fig. 2. In the first conditioning session, the CS was consumed before exposure to the US; thus, consumption of the CS in this period serves as a baseline for intake before any conditioning occurred.

Overall, analyses of effects of the treatment variables on consumption of the CS were assessed by computing a 4 (groups) × 4 (sessions) mixed unweighted means analysis of variance (ANOVA) with repeated measures on the sessions variable. There was no reliable effect for groups ($F(3,19) = 1.49$, $p > 0.25$), but there were reliable effects for sessions ($F(3,57) = 20.98$, $p < 0.001$) and for the interaction of sessions with groups ($F(9,57) = 5.57$, $p < 0.001$).

The simple effects of the groups × sessions interaction were computed to analyze these effects further. The simple effects of groups in the first session reflected there was no reliable difference in fluid consumption by the four groups prior to conditioning ($F(3,19) = 2.29$, $p > 0.11$). The simple effects for each group across the four sessions were computed to clarify interpretation of the sessions × groups interaction. Consumption decreased dramatically for intact animals rotated ($p < 0.001$) or injected with LiCl ($p < 0.001$) reflecting the formation of CTA. However, there was no change in consumption across conditioning sessions for intact animals injected with saline ($p > 0.78$) or AP-lesioned animals injected with LiCl ($p > 0.61$).

These findings indicate that LiCl is an effective US for producing CTA in the squirrel monkey as it is in numerous other species. However, CTA was formed even though no monkey vomited after treatment with this US, confirming that emesis is not necessary for the production of CTA in the monkey. The failure to produce CTA with LiCl in monkeys with AP ablated implies that the AP serves as a chemoreceptive site of action for systemically injected LiCl in the squirrel monkey as it does in rats (Rabin et al. 1983; Ritter et al. 1980; Sutton et al. 1988).

Phase II conditioning

Intact animals injected with saline and AP-lesioned animals injected with LiCl in phase I were both conditioned with motion serving as the US in phase II. Neither of these groups of animals formed CTA in phase I. Of the five intact monkeys previously injected with saline, all but one (which failed to vomit during any of these tests) vomited during each exposure to rotation (latencies to vomit ranged from 4 to 22 min). Three of the seven AP-lesioned monkeys never vomited during con-

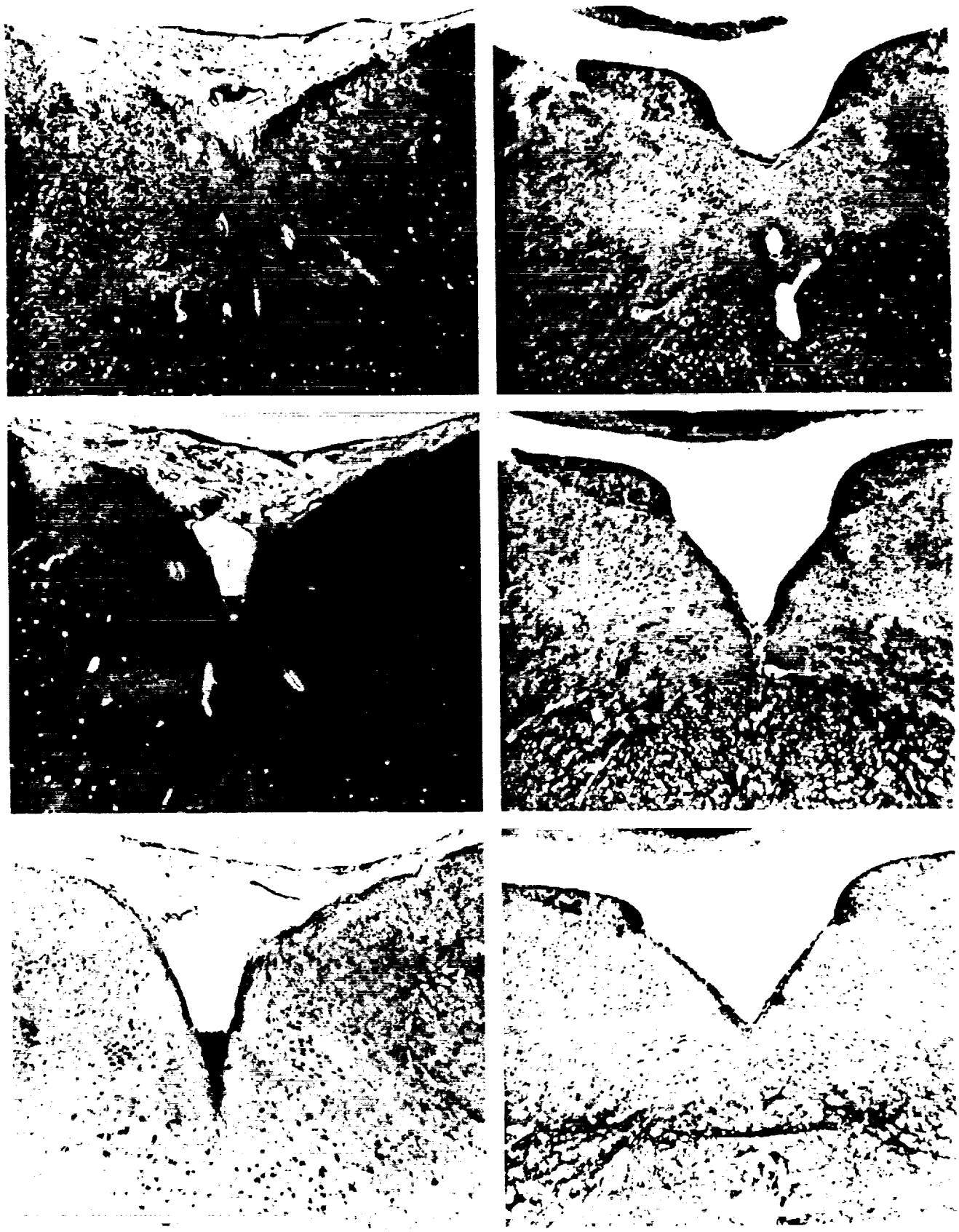

FIG. 1. Sections at three levels of the caudal medulla of squirrel monkeys. Photographs in the right column are for an unoperated control monkey, while those in the left column illustrate the extent of damage in a lesioned monkey.

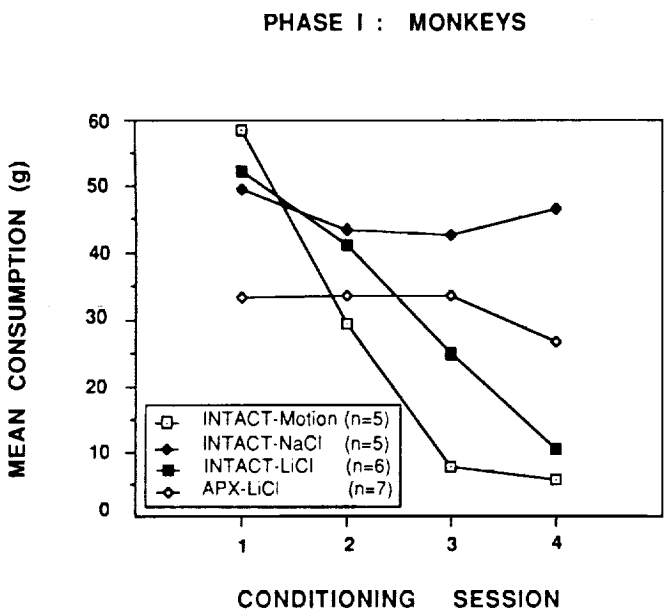

FIG. 2. Average consumption of flavored fluid (CS) by the four groups of monkeys during the first phase of conditioning. Consumption of fluid in conditioning session 1 serves as a baseline measure because fluid was consumed prior to the first treatment with the US. The formation of CTA in intact animals conditioned either with motion or with LiCl is reflected in the progressive decrease in fluid intake in sessions 2 through 4.

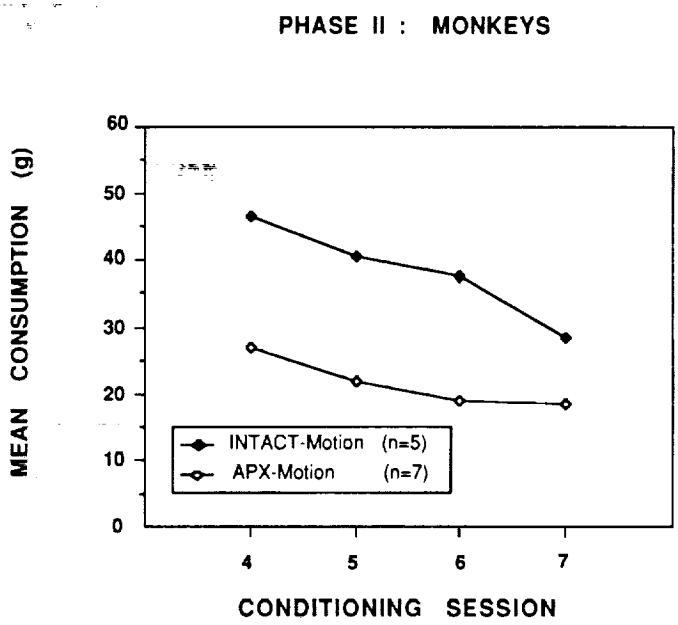

FIG. 3. Average consumption of flavored fluid (CS) by the two groups of monkeys transferred to the second phase of conditioning. Consumption of fluid in conditioning session 4 is reproduced from Fig. 2 and serves as a reference measure for conditioning with motion as the US in phase II. The formation of CTA in intact and lesioned animals conditioned with motion is reflected in the progressive decrease in fluid intake in sessions 5 through 7.

ditioning sessions with rotation as the US, and three vomited during at least two of the conditioning sessions (latencies ranged from 6 to 30 min).

The effects of conditioning with motion in phase II are shown in Fig. 3. The consumption reported for session 4 is the same data shown for session 4 in Fig. 2. These data comprise the appropriate comparison to evaluate conditioning with motion (reflected in sessions 5, 6, and 7) because they reflect the average consumption immediately before motion was used as the US.

Overall analyses of effects of motion on consumption of the CS were assessed by computing a 2 (groups) × 4 (sessions) mixed ANOVA with repeated measures on the sessions variable. This analysis revealed a reliable effect for sessions ($F(3,30) = 3.05$, $p < 0.05$) indicating CTA was formed, but there was no difference between the two groups ($F(1,10) = 1.26$, $p > 0.29$) nor was there a reliable groups × sessions interaction ($F < 1$). Thus, although motion serves as an effective US for conditioning in phase II, the magnitude and rate of formation of the aversion are less than seen with the intact animals in phase I. This apparent reduction in the effectiveness in producing conditioning may be due to repeated exposure to the CS in phase I (Braveman 1975; McLaurin et al. 1963).

These same AP-ablated animals, as a group, failed to form CTA in phase I when LiCl was used as the US indicating the chemoreceptive function of the AP was eliminated by the ablations. Because animals with AP ablated apparently form motion-induced CTA in a manner similar to intact animals, it is implied that the AP plays no crucial role in the formation of motion-induced CTA in the squirrel monkey.

Experiment 2: Cats

Method

Chocolate-flavored milk was used as the CS. During each conditioning session, approximately 100 mL of the CS was available in Petri dishes placed on the floor of a ventilated, clear Plexiglas cage (52 × 23 × 30 cm). Animals were transferred to these cages 10 min before the beginning of the 30-min drinking period to permit acclimation to the test room. The amount of fluid consumed in each drinking period was determined by weighing the Petri dishes, and latencies to retches and vomits were noted.

Cats were assigned to three groups defined by the treatments used as USs in conditioning sessions as follows.

Group 1: Conditioned with xylazine in phase I only ($n = 8$). These animals were injected subcutaneously (s.c.) with 0.66 mg/kg xylazine within 5 min after removal of the CS.

Group 2: Conditioned with saline in phase I followed by xylazine in phase II ($n = 8$). In phase I, the animals were injected subcutaneously with saline (volume equivalent to that for xylazine as in group 1). In phase II (on days 10 and 13) they were injected with 0.66 mg/kg xylazine s.c. as described for group 1 in phase I.

Group 3: AP-lesioned animals conditioned with xylazine in phase I and with motion in phase II ($n = 10$ and $n = 8$, respectively). In phase I, lesioned cats were injected with 0.66 mg/kg xylazine s.c. In phase II (days 10 and 13), eight of the animals (those that did not condition in phase I) were placed in a ventilated Plexiglas cage (50 × 18 × 21 cm) and exposed to sinusoidal vertical linear acceleration (0.6 Hz with either a 30.5 or 61.0 cm excursion) for 60 or 5 min after the first retch/vomit occurred.

Cats were observed continuously for 1 h after injection with xylazine to determine whether vomiting occurred. In the event vomiting had not occurred within the 1st h after injection, periodic checks of the cage were conducted at intervals of approximately 10 min for evidence of vomitus. Similarly, periodic checks of the cage of each animal were made after

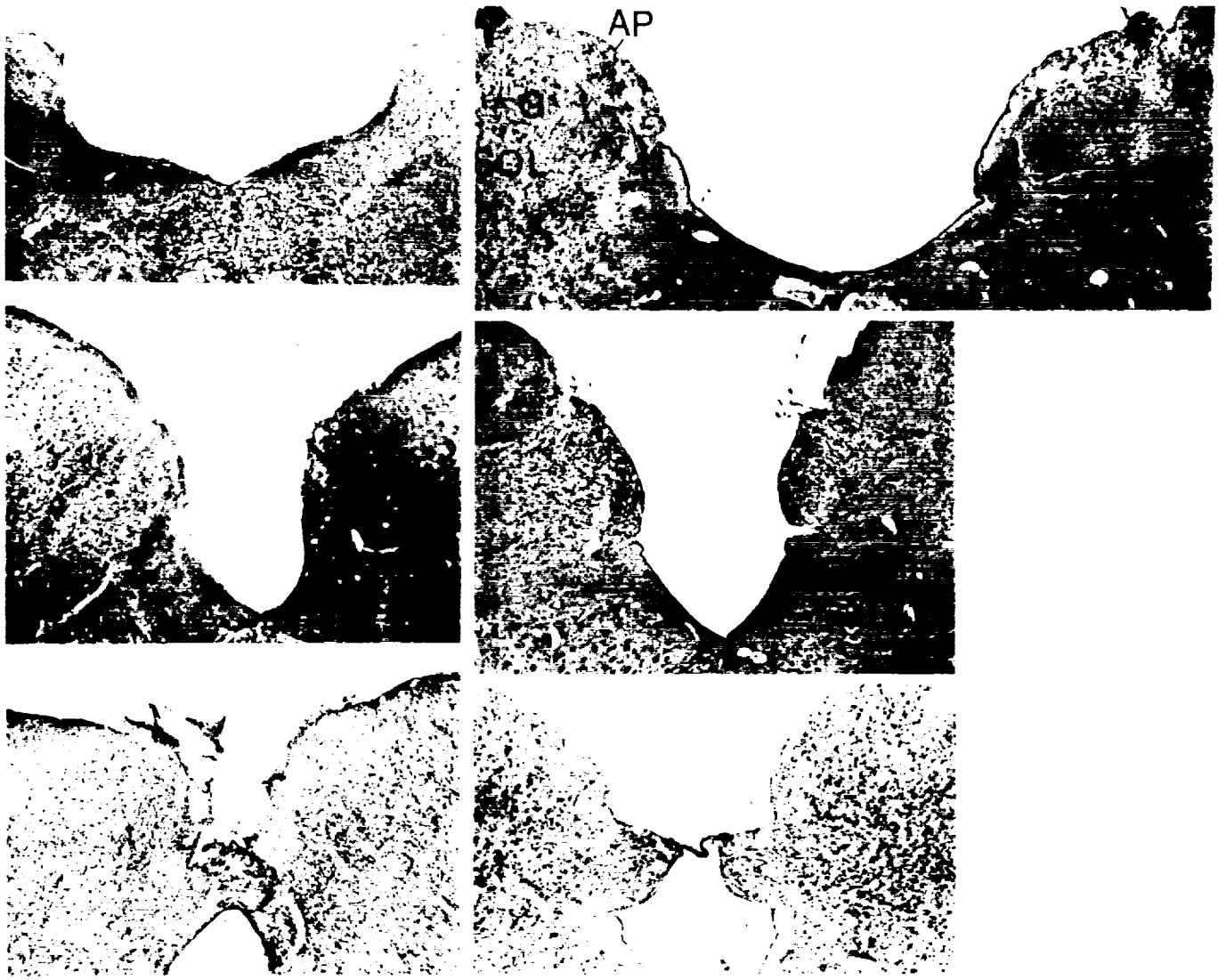

FIG. 4. Sections at three levels of the caudal medulla of cats. Photographs in the right column are for an unoperated control cat, while those in the left column illustrate the extent of damage in a lesioned animal.

motion was terminated. The animals typically appeared relaxed and normal as evidenced by voluntary locomotion by the end of the observation period.

Results

Histology of AP ablations

Sections through the caudal medulla of the AP of an unoperated control (right column) and a lesioned cat (left column) are shown in Fig. 4. As with the monkeys, the AP was destroyed in all animals and varying limited damage occurred to peripostremal structures in some animals. The tractus solitarius was intact in all animals.

Phase I conditioning

Each of the intact cats injected with xylazine vomited in at least one of the conditioning sessions. One cat vomited in one test, three vomited in two sessions, and four vomited in all sessions (latencies to first vomit ranged from 4 to 9 min). None of the AP-lesioned cats vomited when injected with xylazine, and none of the intact cats injected with saline vomited.

The average consumption of chocolate milk by the three groups of cats is shown in Fig. 5. Overall, analyses of effects were assessed by computing a 3 (groups) × 4 (sessions) mixed unweighted means ANOVA with repeated measures on the sessions variable. Reliable effects were indicated for groups ($F(2,22) = 8.42, p < 0.001$), sessions ($F(3,66) = 4.03, p < 0.01$), and the interaction of groups × sessions ($F(6,66) = 10.12, p < 0.001$). Analysis of the simple effects of groups in session 1 reflected there was no reliable difference in the consumption of the CS prior to conditioning ($F(2,22) = 1.52, p > 0.24$).

Further analysis of the groups × sessions interaction was conducted by computing the simple effects of sessions for each of the groups. Consumption of the CS decreased across conditioning sessions for intact cats when xylazine was the US ($p < 0.001$). However, there was no change in consumption of the US across sessions when xylazine was the US for lesioned cats ($p > 0.58$) or when intact cats were injected with NaCl ($p > 0.12$). Thus, the groups × sessions interaction results from the failure of conditioning in intact animals injected with saline and AP-lesioned animals injected with xylazine, contrasted with the dramatic conditioning in intact animals injected with xylazine.

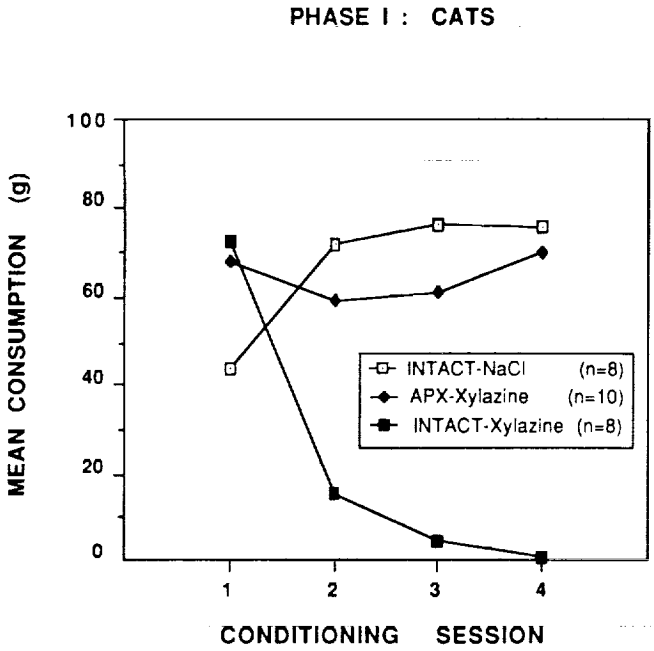

FIG. 5. Average consumption of chocolate milk (CS) by the three groups of cats during the first phase of conditioning. Consumption of fluid in conditioning session 1 serves as a baseline measure because milk was consumed prior to the first treatment with the US. The formation of CTA in intact animals conditioned with xylazine is reflected in the progressive decrease in the intake of milk in sessions 2 through 4.

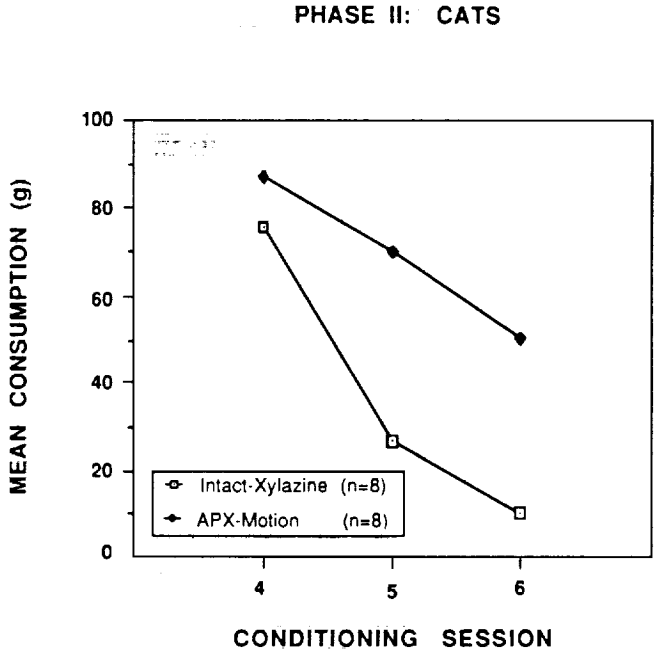

FIG. 6. Average consumption of chocolate milk (CS) by the two groups of cats transferred to the second phase of conditioning. Consumption of milk in conditioning session 4 is reproduced from Fig. 5 and serves as a reference measure for conditioning with the two USs in phase II. The formation of CTA in intact and lesioned animals is reflected in the progressive decrease in fluid intake in sessions 5 through 7.

Phase II conditioning

All of the intact animals injected with saline in phase I (none vomited) and the eight AP-lesioned animals that did not form aversions when injected with xylazine in phase I were conditioned in phase II. Intact animals were injected with xylazine in phase II and lesioned animals were exposed to motion. Seven of the eight intact cats vomited on both conditioning sessions when injected with xylazine, while the remaining cat vomited only following the first injection with xylazine (vomit latencies ranged from 2 to 9 min). Of the eight cats with AP lesions, four failed to vomit during either of the tests when exposed to motion, three vomited during one of the two tests, and one cat vomited during both tests (latencies ranged between 3 and 4 min).

The effects of conditioning with these USs are shown in Fig. 6. The consumption reported for the intact animals in session 4 in this figure are the same data shown for this day in Fig. 5. The data shown in session 4 for lesioned animals report the average for the 8 cats transferred to phase II rather than the 10 cats conditioned in phase I.

A 2 (groups) × 3 (sessions) mixed ANOVA with repeated measures on the sessions variable was used for an overall analysis. Reliable effects were reflected for sessions ($F(2,28) = 21.87, p < 0.001$), indicating aversion was produced, and for groups ($F(1,14) = 8.75, p < 0.01$), indicating stronger aversion in cats injected with xylazine. The interaction of groups × sessions was not reliable ($F(2,28) = 2.42, p > 0.11$). The simple effects of sessions were computed to analyze the main effect for groups further. There was no difference in the consumption of the groups on day 4 ($p > 0.35$), but consumption by intact cats injected with xylazine was less than consumption by lesioned cats rotated on both day 5 ($p < 0.02$) and day 6 ($p < 0.007$). Thus, the stronger aversion for the cats injected with xylazine was present on the first conditioning trial.

Little difference is apparent in the magnitude of xylazine-induced aversions in intact animals conditioned in phases I and II. Thus, no CS preconditioning exposure effect occurred when multiple injections of xylazine served as the US for cats. No appropriate control group was included to evaluate a potential CS preconditioning exposure effect when motion was used as the US with cats. It is apparent, however, that elimination of the chemoreceptive function of the AP in cats as evidenced by failure of xylazine to produce CTA in phase I did not prevent the production of motion-induced CTA. Thus, as in squirrel monkeys, integrity of the AP is not necessary for the production of either vomiting or CTA in cats.

Discussion

The results of this study confirm a chemoreceptive function of AP for xylazine-induced vomiting in cats (Colby et al. 1981). In addition, a chemoreceptive function for the AP in the production of pharmacologically induced CTA is indicated for both monkeys and cats. After ablation of the AP, xylazine was not an effective US for inducing CTA in 8 of the 10 cats tested. The occurrence of CTA in two of the AP-ablated cats indicates a disassociation of CTA from vomiting, because neither of these cats vomited in response to the xylazine injections, which served as the US to produce CTA. In squirrel monkeys, CTA was not produced by injections of LiCl after the AP was destroyed. Thus, the well-documented role of AP in lithium-induced CTA in the rat (Rabin et al. 1983; Ritter et al. 1980; Sutton et al. 1988) is extended to the squirrel monkey.

TABLE 1. Possible outcomes when two criterion measures of illness are compared. In this example, criterion measure 1 is vomiting and criterion measure 2 is conditioned taste aversion (CTA). "Decision" labels in the table indicate the validity of measurement based on criterion measure 2 alone

Criterion measure 1 (vomiting)	Criterion measure 2 (CTA)	
	Present (+)	Absent (−)
Present (+)	Both measures +	Measure 1 + Measure 2 −
	Decision: correct detection ("hit")	Decision: false rejection ("miss")
Absent (−)	Measure 1 − Measure 2 +	Both measures −
	Decision: false detection ("false alarm")	Decision: correct rejection

Motion-induced vomiting was produced in both squirrel monkeys and cats after ablation of the AP confirming previous reports by Wilpizeski et al. (1986) for the squirrel monkey and Borison and Borison (1986) for the cat. The production of motion-induced CTA in AP-ablated cats and squirrel monkeys shown not to condition with pharmacological agents functioning via the AP demonstrates that this form of CTA can occur in the absence of the chemoreceptive function of the AP. Thus, it appears that neither vomiting nor CTA induced by motion depend on a humoral factor operating via a system requiring an intact AP (Crampton and Daunton 1983; Contrucci and Wilpizeski 1985).

Because CTA and vomiting can be elicited in cats and squirrel monkeys following elimination of the chemoreceptive function of AP for pharmacological agents, it appears that motion-induced vomiting and CTA can occur independently of the chemoreceptive function of the AP. Both responses can be produced by two (or more) systems, but it is not clear from our present knowledge whether they are produced by the same system. It has been suggested from a review of pharmacological studies that vomiting may be a sufficient but not a necessary condition for the production of CTA (Gamzu et al. 1985). However, it has been shown that CTA may not occur in some squirrel monkeys when rotation is terminated immediately after vomiting occurs (Wilpizeski and Lowry 1987; Wilpizeski et al. 1987, Figure 4B). Thus, vomiting is not a sufficient condition for the development of CTA in the squirrel monkey.

Disassociation of vomiting from CTA has been interpreted to indicate that nausea is the putative US producing CTA. Roy and Brizzee (1979) proposed using CTA to assess subemetic symptoms of motion sickness in the squirrel monkey. Wilpizeski and Lowry (1987) proposed that nausea and vomiting are independent processes, and they developed a two-factor theory of motion sickness in squirrel monkeys where CTA is used to assess the presence of nausea. They assume that (*i*) nausea produces CTA and (*ii*) vomiting is not a sufficient condition for producing CTA. If nausea and vomiting are independent, then animals can theoretically be categorized into one of the following four possible combinations: (*i*) nauseous and vomiting, (*ii*) nauseous without vomiting, (*iii*) vomiting without nausea, and (*iv*) not nauseous and not vomiting.

Riley and Tuck (1985) have addressed the interpretation of studies using CTA to assess drug toxicity by using the signal detection framework. To do this, individual instances of the presence or absence of illness assessed by two independent measures (i.e., CTA and vomiting) are categorized into a 2 × 2 contingency table (see Table 1). When results of these measures agree, each case is scored as either a "hit" (correct identification) or a "correct rejection." When results of these measures disagree, each case is scored as either a "false alarm" (false detection) or a "miss" (false rejection).

According to this contingency table analysis, the possible combinations of nausea and vomiting are categorized as hit (nauseous and vomiting), false alarm (nauseous without vomiting), miss (vomiting without nausea), and correct rejection (not nauseous and not vomiting).

To apply this analysis to these data, animals were categorized based on their CTA/vomiting responses. Contingency table percentages were computed on data from these experiments using all of the AP-lesioned animals. CTA was scored as having been produced when consumption of fluid on the test day was $<75\%$ of consumption on the initial (baseline) day. Percentages of animals in a category were computed for the first and last conditioning sessions to evaluate whether the ratio of animals in each category changed as more conditioning sessions were used. Percentages computed from combined data in phases I and II ($n = 32$) are shown in Table 2. The increase in hit rate and decrease in miss rate from the first to the last conditioning test reflects the greater agreement of the two measures for predicting motion sickness as more conditioning tests are used. If nausea is estimated as the percentage of animals in the false alarm and hit categories, it increases from 34 to 50% by the final assessment. Notice, however, that the percentage of cases reflected by false alarms (animals assumed to be nauseated only) is invariant.

Percentages based on data for phase I and phase II separately are shown in Table 3. Hits and misses could not occur in the analysis for phase I because no animal vomited when injected with drugs. Some animals vomited in phase II when exposed to motion, so all possibilities can occur when phase II data are categorized. The false alarm rate is again invariant from the first to the last conditioning test in data for both phases. However, when the US elicited vomiting on some tests in phase II, the miss rate decreased from the first to the last conditioning test accompanied by an increase in the hit rate, again indicating improved agreement between the measures.

Interpretation of the sum of hits and false alarms as an accurate measure of nausea is based on the assumption that nausea is reliably and accurately reflected by CTA. This may be so, but skepticism has been proposed (Gamzu et al. 1985),

TABLE 2. Percentages of animals in the four possible categories defined by agreements and disagreements when the results of CTA and vomiting are used to identify motion sickness. Percentages computed on combined data (n = 32) from the two conditioning phases

	Conditioning test	
Category	First	Last
Miss	23	3
False alarm	27	27
Correct rejection	43	47
Hit	7	23

TABLE 3. Percentages of animals in the four possible categories defined by agreements and disagreements when the results of CTA and vomiting are used to identify motion sickness. Percentages for the two conditioning phases are computed separately

	Conditioning test	
Category	First	Last
Phase I (n = 17)		
Miss	0	0
False alarm	35	35
Correct rejection	65	65
Hit	0	0
Phase II (n = 15)		
Miss	47	7
False alarm	20	20
Correct rejection	20	27
Hit	13	47

and it remains to be tested directly. The apparently invariate rate of false alarms may indicate one way to perform such a test. If animals that are nauseous only are in fact identified as cases of false alarms, then those animals would form an appropriate subgroup for testing the correlation of CTA with an independent measure of nausea. No reliable measure of nausea for conducting such an analysis has been demonstrated. Tachygastria may be one candidate for such an analysis. Because tachygastria appears to precede nausea in humans, a perfect correlation between the production of motion-induced CTA in animals identified as nauseous (i.e., false alarm cases) and tachygastria in those same animals would imply nausea is the putative US for CTA. Another possible measure is the disruption of electrical control activity cycling associated with retrograde contractions of the gut, which has been shown to be associated with vomiting in dogs (Lang et al. 1986). If either of these measures should prove to be appropriate, however, it may prove to be more useful than CTA for assessing nausea.

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Current status: animal models of nausea

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SUMMARY

The advantages, and possible benefits of a valid, reliable animal model for nausea are discussed, and difficulties inherent to the development of a model are considered. A principle problem for developing models arises because nausea is a subjective sensation that can be identified only in humans. Several putative measures of nausea in animals are considered, with more detailed consideration directed to variation in cardiac rate, levels of vasopressin, and conditioned taste aversion. Demonstration that putative measures are associated with reported nausea in humans is proposed as a requirement for validating measures to be used in animal models. The necessity for a "real-time" measure of nausea is proposed as an important factor for future research; and the need for improved understanding of the neuroanatomy underlying the emetic syndrome is discussed.

Les modèles animaux dans l'étude de la nausée: état de la question

Résumé: Les avantages et bénéfices possibles d'un modèle animal dans l'étude de la nausée sont discutés; les difficultés inhérentes au développement de ce modèle sont abordées. Un problème de principe pour développer de tels modèles (animaux) réside dans le fait que la nausée est une sensation subjective qui ne peut être identifiée que chez l'homme. Plusieurs indices pouvant permettre de détecter la nausée chez l'animal sont présentés, en insistant plus particulièrement sur les variations du rythme cardiaque, le taux sérique de vasopressine et le comportement de révulsion alimentaire conditionné. La démonstration que ces indices sont aussi associés à une nausée avérée chez l'homme est proposée comme une condition de validation de ces indices chez l'animal. La nécessité d'objectiver la nausée en temps réel est proposée comme un facteur important pour les futures recherches. Le besoin d'une meilleure connaissance neuroanatomique des circuits qui sous-tendent le syndrome émétique est discuté.

INTRODUCTION

Nausea generally is not life threatening, but it can have significant negative impact in clinical procedures (Wetchler, 1991) and chronic nausea may lead to a marked reduction in the quality of life (Stewart, 1991). Patients with predisposition to prolonged gastric emptying or those undergoing laparoscopy are at high risk for nausea and intractable vomiting when anesthesia

or sedation are required (Kapur, 1991). Nausea and vomiting also are severe side effects of chemotherapy and contribute importantly to noncompliance with treatment regimens, particularly in adolescents (Zelter *et al.*, 1991). In addition, anticipatory nausea is a significant problem with up to one fourth of pediatric patients undergoing chemotherapy (Dolgin *et al.*, 1985).

A valid animal model of nausea would contribute importantly to the study of the neural and physiological systems involved in this state. Miller and Kucharczyk (1991) noted that the lack of such a model has hampered investigation of both the etiology of nausea and the relationship of nausea to vomiting. Development and verification of an animal model of nausea is difficult, however, for both practical and theoretical reasons. Practical difficulties arise because there is no accepted physiological method for identifying the subjective state of nausea in animals, or for that matter in humans. Self-reports of nausea are accepted in humans, but there are no reliable, direct measures of either the presence or degree of this state. Several putative measures of nausea have been suggested, but as is discussed below, none has been convincingly demonstrated as reliable, workable, and valid. This absence of direct measures of nausea creates a significant problem for the validation of animal models.

Development of animal models is complicated further by the fact that species differences in this response are unknown. Vomiting, the culminating event of the emetic syndrome, can be identified directly and is widespread in the animal kingdom. However, the wide variety of stimuli that elicit, or fail to elicit vomiting in various animals (Corcoran, Fox, & Daunton, 1990; Daunton, 1990; King, 1990) might imply that variations are to be expected in nauseogenic responses as well. The observation of vomiting may *not necessarily* indicate that nausea is, or has been, present. Nausea and emesis are not inextricably linked in humans (Harm, 1990) and there is no *a priori* reason to believe that they would be linked in a possible animal model.

Current theoretical interpretations of the neurophysiological mechanisms of vomiting indicate another problem for the development of models. The traditional concept that effector activation of vomiting is coordinated by a localized group of neurons, or vomiting "center" (Borison & Wang, 1949) is now questioned (Miller & Wilson, 1983a). Current interpretations of possible mechanisms for the nausea-emetic syndrome propose that this state may be mediated via multiple pathways (Miller & Wilson, 1983b) rather than a single emetic center. Such schemes may involve predominant pathways for a given emetic stimulus or species (Harding, 1990) or a hierarchical cascade of effector systems that may vary for different animals (Lawes, 1991). Evolutionary development of multiple pathways provides diverse opportunities for variation in the mechanisms of nausea and vomiting among species.

DETECTION OF NAUSEA WITH INDIRECT MEASURES

Following the suggestion of Borison and Wang (1953), indirect measures for nausea have been chosen to reflect autonomic responses thought to accompany this state. Several responses have been used as prodromal signs of nausea. Applications of this approach range from the development of formal rating scales to the reporting of individual responses thought to be prodromal symptoms of sickness. Demonstration that a putative measure is associated with reported nausea in humans is crucial to the validation of measures to be used in animal models.

Rating scales are based on the concept that various autonomic responses (e.g., increased

salivation, disruptions of cardiac rhythm, defecation) that often precede vomiting are associated with nausea. An implicit assumption of this approach is that autonomic signs of sickness reflect an underlying serial process progressing from mild disturbance through nausea toward frank vomiting. Formal rating scales were developed for human studies of motion sickness (Graybiel, Wood, Miller, & Cramer, 1968) so that stimulation could be terminated prior to frank vomiting, and to provide a graded measure of sickness. A scale of one form or another, and self-reported nausea, are used in virtually all studies of motion sickness in man. Analogous scales have been developed to assess the development of motion sickness in cats (Suri, Crampton, & Daunton, 1979), squirrel monkeys (Igarashi *et al.*, 1983) and chimpanzees (Meek, Graybiel, Beischer, & Riopelle, 1962). Several observations indicating that the individual responses comprising rating scales fail to reflect a serial, unitary emetic mechanism in motion sickness are reviewed by Daunton (1990). This lack of evidence of a serial mechanism in motion sickness raises serious concerns regarding the use of rating scales to assess the development of sickness (i.e., nausea) in animal models.

A wide variety of individual responses have been used to assess sickness in animals. Some of these are included in typical rating scales, while others are not. A partial summary of the responses used with various species is outlined in Table 1. Several of these responses (e.g., reduced activity or food intake, defecation, pica) are observed in humans during activation of the emetic syndrome and were adopted as measures to be used with species that do not possess a complete emetic reflex. The use of such species (e.g., the rat) is motivated, in part, by the utility of these standard laboratory animals for physiological investigations. Although the use of these species to assess emetic mechanisms is questionable, these measures continue to receive consideration as multiple, or supplemental indices of sickness (Ossenkopp & Ossenkopp, 1985).

Table 1. Several putative measures of sickness (nausea) and the species that have been tested with each measure.

MEASURE	SPECIES
Arginine Vasopressin (AVP)	human, monkey, cat, rat
Burrowing and Backing	ferret
Cardiac Rhythm	human, squirrel monkey
Conditioned Taste Aversion (CTA)	human, squirrel monkey, cat rat, guinea pig, mouse
Defecation	cat, ferret, rat
Gastric Rhythms	human, dog
Pica	human, rat
Reduced Activity	ferret, rat
Reduced Intake of Food or Water	human, rat
Skin Color Changes	human, squirrel monkey

POSSIBLE MARKERS FOR NAUSEA

Some measures have been adopted specifically to assess nausea. Three of these are discussed in the following sections.

Cardiac Rhythm

A relationship between cardiac irregularity and the emetic reflex has long been suspected (Crittenden & Ivy, 1933). Ishii *et al.* (1987) used beat-to-beat variation in cardiac rhythm to assess autonomic nervous system effects related to "motion sickness" induced by vestibulo-visual conflict in squirrel monkeys. Monkeys were secured in a primate chair to avoid movement artifacts. Variation in beat-to-beat intervals increased immediately prior to vomiting, perhaps reflecting nausea. Two effects indicate these changes could arise from altered parasympathetic activity: injections of atropine reduced variations in animals in control conditions and counteracted increased variation in animals subjected to vestibulo-visual conflict. Demonstration of a relationship between these changes and other possible indices of nausea (i.e., see changes in AVP discussed below) should be considered to investigate these effects further. Cardiac arrhythmia can be processed in real time by computer analysis, and thus could provide a direct, "on-line" measure of parasympathetic activity in conditions when effects from other factors such as stress or blood pressure can be controlled or eliminated.

Release of Vasopressin

The level of systemic vasopressin (AVP) has been investigated as a possible objective marker for nausea or activation of emetic pathways. AVP is elevated during nausea and after vomiting in man (e.g., Koch *et al.*, 1990; Miaskiewicz, Striker, & Verbalis , 1989; Rowe *et al.*, 1979), and after vomiting in cats (Fox *et al.*, 1987) and monkeys (Verbalis, Richardson, & Striker, 1987). However, most examinations of the relationship between nausea and AVP have been correlational in nature, and there is no definitive explanation of the physiological events underlying it. AVP is excitatory to neurons of the chemoreceptive trigger zone (Carpenter, Briggs & Strominger, 1984), but an emetic effect of AVP is not well documented. Infusion of AVP has produced emesis in humans (Thomford & Sirinek, 1975), but infusion also has failed to produce emesis in man (Williams *et al.*, 1986) and in cats (Fox, unpublished data). Explanation of any cause and effect relationship between nausea and elevated levels of AVP is crucial to the use of AVP as a marker for nausea in an animal model.

Two recent studies have addressed the relationship between nausea and AVP. Koch *et al.* (1990) induced malaise using illusory self-motion. Koch (1991) notes that both reported nausea and the release of AVP in this study are related to gastric arrhythmia, and proposes that either of two possible sequences, **arrhythmia** ---> **nausea** ---> AVP release or **arrhythmia** ---> AVP release ---> **nausea**, are possible. Miaskiewicz *et al.* (1989) stimulated nausea and vomiting in humans by injection of cholecystokinin octapeptide (CCK). Doses of CCK that caused epigastric cramping and mild visceral discomfort were associated with increased levels of AVP, however, these effects occurred without reports of nausea. This result was interpreted as suggesting that AVP secretion can occur with minor visceral malaise even prior to nausea or emesis, perhaps indicating that secretion of AVP precedes nausea.

The efficacy of AVP as a marker for nausea has not been demonstrated convincingly for animal models. Two factors should be considered prior to using AVP to identify nausea or the activation of emetic pathways. First, large individual differences in the range of the AVP response have been observed in studies with humans (Edwards, Carmichael, Baylis, & Harris, 1989; Koch *et al.*, 1990; Miaskiewicz *et al.*, 1989), monkeys (Verbalis *et al.*, 1987), and cats (Fox *et al.*, 1987). Second, not all cases of nausea are associated with AVP secretion. A dissociation of AVP and nausea was shown when nausea, induced by rapid food intake, failed to be associated with elevated AVP (Miaskiewicz *et al.*, 1989). Neither is the association

between emesis and AVP secretion obligatory.since elevation of AVP fails to occur in man when emesis is induced with ipecacuanha syrup (Nussey *et al.*, 1988).

Technological issues also complicate the use of AVP as a marker of nausea in animal studies. Assays for AVP require blood volumes which prohibit serial sampling in small animals. This is a serious problem with very small animals like shrews, where it may not be possible to obtain even single samples without producing changes in blood pressure or plasma osmolality. Obtaining repeated samples from cats may impact other indices of general stress such as cortisol (Fox *et al.*, 1987). In addition, significant processing is required to conduct the assay, so assessment of AVP cannot provide an "on-line" index of the nauseous state.

Conditioned Taste Aversion

The avoidance of flavored substances consumed just prior to the onset of sickness (a conditioned taste aversion, or CTA) was first demonstrated in the laboratory by Garcia and colleagues (e.g., Garcia & Ervin, 1968). Because many of the stimuli used to induce CTA in early experiments produce gastrointestinal distress or nausea, CTA was thought to be mediated by neural mechanisms important to the emetic syndrome. The observation of CTA in patients made nauseous while undergoing chemotherapeutic (Bernstein, 1985; Bernstein & Webster, 1980) or radiation treatments (Smith et al., 1984) for cancer provides further support for this position.

Few direct, systematic evaluations of the assumption that visceral distress, or nausea promote CTA have been conducted. In a retrospective evaluation conducted by reviewing the literatures on vomiting and CTA, Grant (1987) argued that if nausea or other pre-emetic components of the emetic syndrome are responsible for CTA, then CTA should depend on neural structures important to the emetic syndrome (assessed by vomiting). Available studies that investigated whether neural pathways important to emesis also are important to the production of CTA neither confirm nor reject convincingly a role for emetic structures in CTA. Some blood-borne agents such as lithium chloride (McGlone, Ritter, & Kelly, 1980; Rabin, Hunt, & Lee, 1983), copper sulfate (Coil & Norgren, 1981), or xylazine (Fox, Corcoran & Brizzee, 1990) that produce CTA do depend on the area postrema. The disruptive effects of AP lesions on CTA induced by toxins are sufficiently reliable that both lithium chloride (Sutton, Fox, & Daunton, 1988) and scopolamine methyl nitrate (Ossenkopp, 1983) have been used to screen for the completeness of AP lesions in rats. For other agents, however, CTA and vomiting do not depend on the same neural mechanisms. Morphine, for example, produces vomiting via the area postrema (Borison *et al.*, 1962) but produces CTA via the periaquaductal gray (Blair & Amit, 1981).

Several factors require that conclusions from Grant's review be made with care. Grant acknowledged that emetic circuitry is incompletely understood and that most research on CTA has been conducted with rats while that on emetic mechanisms has been on cats, dogs, and monkeys. (Ferrets must now be added to the list of animals used to study emetic mechanisms). Because there are species differences in sensitivity to emetic treatments, cross-species comparisons can be difficult. However, cross-species comparisons are required because the relationship between CTA and the emetic syndrome has been investigated directly in very few studies (Fox *et al.*, 1990; Rabin *et al.*, 1986; Roy & Brizzee, 1979; Wilpizeski *et al.*, 1985). These studies generally show that vomiting does not predict the formation of CTA precisely. CTA may occur without vomiting and vomiting may occur without CTA being produced. Additional studies directly assessing CTA and the emetic syndrome in the

same species (or animals) will be required to clarify whether the emetic syndrome and CTA share common neural circuitry.

Because CTA is a learned response, several control conditions for the study of learning mechanisms are required when this measure is used (Fox, 1990). Procedures to eliminate psuedoconditioning and other artifactual effects that could be incorrectly interpreted as CTA can significantly increase the effort and cost involved in using this measure. The possibility that exposure to the emetic stimulus prior to testing could reduce the strength of CTA, and thus lead to incorrect inferences about the effect of the stimulus, can restrict methods for conducting experiments.

CONCLUSIONS

Development of a valid animal model of nausea requires the identification of motor, hormonal, neural, or behavioral events that are associated with nausea. Because nausea is a subjective state that can be identified only in man, potential measures for animal models must be based on demonstrations that the same measures reliably identify nausea in man. Thus, a coordinated research strategy that integrates information from studies in humans and animals is required. Confidence in a given measure could be enhanced by the accumulation of convergent validation data from multiple assessments (i.e., motor and hormonal).

Each of the potential measures of nausea discussed above is affected by one or more detrimental factors. All of these potential measures require better validation. Technical requirements for assaying systemic AVP can produce general stress effects, or even prohibit application of the measure in very small animals. Concern about neural circuitry crucial to CTA, requirements for control procedures, and the observation that nausea and/or vomiting can occur independently of CTA indicate reservations about this measure. In addition, investigation of the effects of antiemetic drugs on CTA have produced positive (Coil *et al.*, 1978) and negative (Goudie *et al.*, 1982) results, and some of the compounds used as antiemetics can produce CTA themselves (i.e., scopolamine).

Neither AVP nor CTA can provide a real-time assessment of sickness. Variation in cardiac rhythm could provide real-time assessment, but this measure needs to be validated with additional studies. The source or type of other possible measures for nausea are not readily forthcoming. Incomplete characterization of the neuroanatomy that underlies the emetic syndrome complicates identification of prodromal signs sufficiently independent of emesis that they might serve as measures of other components of the syndrome. Thus, recent research has characterized gastrointestinal precursors of vomiting (Lang *et al.*, 1986; Lang, Sarna, & Condon, 1986) but has not provided a clear candidate for an index of nausea. Gastric relaxation could be a candidate (Andrews & Wood, 1988; Hulse & Patrick, 1977; Willems & Lefebvre, 1986) but this response also occurs as part of the normal sequence of feeding (Young & Deutsch, 1980), and not all forms of gastric distention produce nausea (Miaskiewicz *et al.*, 1989). If this response is related to nausea, an explanation of why it leads to the sensation of nausea in some instances but not in others is required.

Improved understanding of the neurocircuitry of the emetic syndrome is a primary requirement for the development of a model for nausea. The neural mechanisms underlying nausea will not be identified until the events that converge to elicit vomiting are described more fully. Improved understanding of interrelationships between prodromal signs of vomiting and identification of the mechanism coordinating the neural activity that produces the complicated

pattern of motor events leading to expulsion would be very beneficial. At the present time there is little evidence indicating whether the mechanisms underlying nausea should be sought in central or peripheral sites.

The species best suited for a model is not obvious. Each species traditionally used to study emetic mechanisms (dogs, cats, ferrets and monkeys) has advantages for specific purposes. The extensive knowledge of neural, receptor, and gastrointestinal mechanisms in these animals is invaluable. But other tractable animals that readily can be bred for purpose to insure availability at reasonable cost would be advantageous. The ferret has been very useful in recent years, and the house shrew, *Suncus murinus*, is a relatively new candidate on the scene that shows promise (Matsuki *et al.*, 1988: Ueno, Matsuki, & Saito, 1987; Ueno, Matsuki, & Saito, 1988). The shrew is very small for some procedures, such as blood assays and instrumentation, but this small size is an advantage for housing and testing of chemical agents that are difficult to produce in large quantities. If detailed description of neuroanatomy and physiology are forthcoming this may prove to be a useful animal. Certainly the rat is not an ideal model. Thorough knowledge of anatomy and physiology are a valuable asset, but the lack of an emetic reflex leads to complex issues of species differences that complicate understanding.

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