

1994017432 ;

N94-21905

49.19

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

M. Corcoran*, R. Fox*, K. Brizzee*, G. Crampton*, and N. Daunton* (SPON: J. Zabara). NASA Ames Research Center, Moffett Field, CA. 94035; San Jose State Univ., San Jose, CA. 95192; Delta Regional Primate Center, Covington, LA. 70433; Wright State Univ., Dayton, OH. 45435.

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.

330

PRECEDING PAGE BLANK NOT FILMED

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

ROBERT A. FOX, B.A., PH.D., ANDREA H. LAUBER, B.A., M.A., NANCY G. DAUNTON, M.A., PH.D., MARILYN PHILLIPS, B.A., and LINDA DIAZ, B.A., M.A.

San Jose State University, San Jose, California 95192 and NASA-Ames Research Center, Moffett Field, CA 95035

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 animal 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 preliminary 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

Authors R. A. Fox, A. H. Lauber, M. Phillips, and L. Diaz are with San Jose State University, and N. G. Daunton is with NASA-Ames Research Center.

This manuscript was received for review in August 1983. The revised manuscript was accepted for publication in March 1984.

OFF-VERTICAL ROTATION—FOX ET AL.

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 \times 24.0 cm long \times 12.8 cm high) to form three compartments (6.5 cm \times 8.0 cm \times 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 $\cdot\text{L}^{-1}$ 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

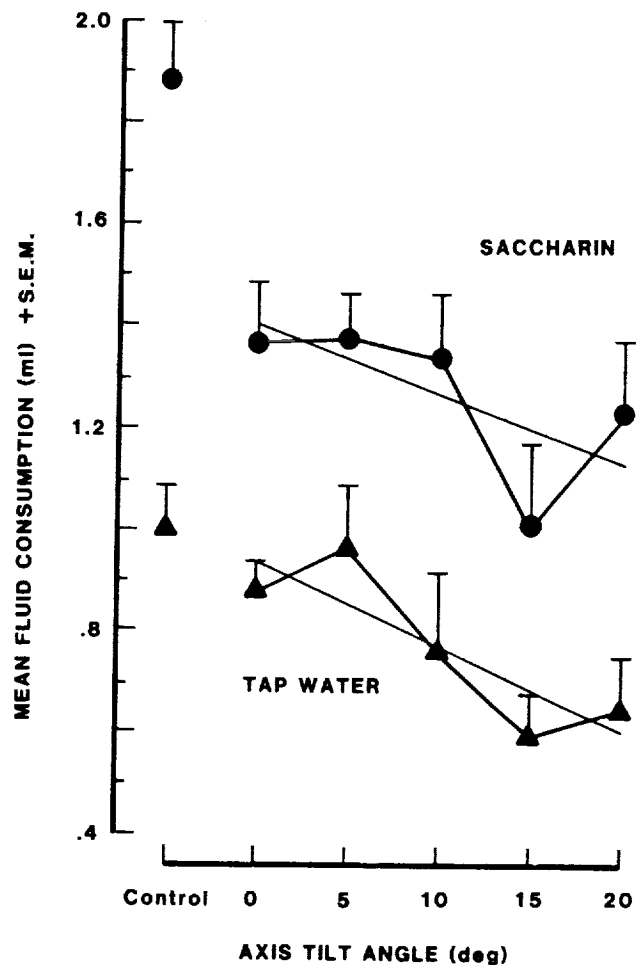


Fig. 1. Average consumption of saccharin water (3 d after conditioning) and of tap water (immediately after rotation) by the control subjects and by the animals rotated at various angles of tilt from earth-vertical.

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

OFF-VERTICAL ROTATION—FOX ET AL.

3. Graybiel, A., and E. F. Miller II. Off-vertical rotation: A convenient precise means of exposing the passive human subject to a rotating linear acceleration vector. *Aerospace Med.* 41:407-410; 1970.
4. Graybiel, A., C. D. Wood, E. F. Miller, and D. B. Cramer. Diagnostic criteria for grading the severity of acute motion sickness. *Aerospace Med.* 39:453-455; 1968.
5. Green, L., and H. Rachlin. Learned taste aversions in rats as a function of delay, speed, and duration of rotation. *Learn. Motiv.* 7:283-289; 1976.
6. Haroutunian, V., D. C. Riccio, and D. P. Gans. Suppression of drinking following rotational stimulation as an index of motion sickness in the rat. *Physiol. Psychol.* 4:467-472; 1976.
7. Igarashi, M., H. Isago, T. O-Uchi, W. B. Kulecs, J. L. Homick, and M. F. Reschke. Vestibular-visual conflict sickness in the squirrel monkey. *Acta Otolaryngol.* 95:193-198; 1983.
8. Miller, E. F. II, and A. Graybiel. A provocative test for grading susceptibility to motion sickness yielding a single numerical score. *Acta Otolaryngol.* [Suppl.]:274-296; 1970.
9. Mitchell, D., M. L. Krusemark, and E. Hafner. Pica: A species relevant behavioral assay of motion sickness in the rat. *Physiol. Behav.* 18:125-130; 1977.
10. Money, K. E. Motion sickness. *Physiol. Rev.* 50:1-39; 1970.
11. Ossenkoop, K.-P., and N. L. Frisken. Defecation as an index of motion sickness in the rat. *Physiol. Psychol.* 10:355-360; 1982.
12. Roy, M. A., and K. R. Brizzee. Motion sickness-induced food aversions in the squirrel monkey. *Physiol. Behav.* 23:39-41; 1979.

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, it 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 suggests 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.

ACKNOWLEDGMENTS

Funds for this study were provided under Interchange Agreement NCA 2-OR-675-801 by Ames Research Center, NASA, Moffett Field, CA, and by NIH Grant S06RR08192-02 to Robert A. Fox.

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

REFERENCES

1. Braun, J. J., and H. McIntosh, Jr. Learned taste aversions induced by rotational stimulation. *Physiol. Psychol.* 1:301–304; 1973.
2. Fox, R. A., and N. G. Daunton. Conditioned feeding suppression in rats produced by cross-coupled and simple motions. *Aviat. Space Environ. Med.* 53:218–220; 1982.