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The Effects of preconditioning exposure to rotation on conditioned taste aversion when rotation is used as the unconditioned stimulus

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Reis, Clifford, M.A.

San Jose State University, 1989

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THE EFFECTS OF PRECONDITIONING EXPOSURE
TO ROTATION ON CONDITIONED TASTE AVERSION
WHEN ROTATION IS USED AS THE UNCONDITIONED STIMULUS

A Thesis
Presented to
the Faculty of the Department of Psychology
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

by
Clifford Reis
August, 1989

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**The Effects of Preconditioning Exposure
to Rotation on Conditioned Taste Aversion
When Rotation is Used as the Unconditioned Stimulus
Clifford Reis
San Jose State University**

Running head: PRECONDITIONING EXPOSURE TO ROTATION

Abstract

The US preconditioning effect was investigated using rotation as the US in a conditioned taste aversion (CTA) paradigm. Animals exposed to a stimulus prior to conditioning with that stimulus as an US typically display weaker learning after conditioning. Four groups of male Long-Evans rats received either 0, 1, 3 or 5 preconditioning exposures to off-vertical rotation and were then trained to avoid a saccharin CS using rotation-induced illness. In addition to a 10-min single bottle test, long-term preference was measured with a 10-day 2-bottle test. Previous studies involving pharmacological USs revealed a systematic, progressive decrease in the magnitude of CTA as the number of exposures increased. In this study, groups receiving 0 or 1 preconditioning exposure formed similar aversions to the saccharin solution while those groups receiving 3 or 5 preconditioning exposures demonstrated no CTA. It is suggested that animals respond differently to rotationally-induced as opposed to pharmacologically-induced illness and that parametric studies involving rotation are lacking.

**The Effect of Preconditioning Exposure to Rotation
on Conditioned Taste Aversion when
Rotation is used as the Unconditioned Stimulus**

The conditioned taste aversion (CTA) paradigm invokes a unique form of learning involving the association of gustatory cues with internal malaise. After ingesting a novel tasting substance, the conditioned stimulus (CS), the animal is made ill via a toxic agent, the unconditioned stimulus (US), and the resulting conditioned response is such that the animal avoids the CS in future tests. Conditioning can occur in one trial, it is an easily observed phenomenon, and the variables affecting the strength of conditioning can be manipulated easily.

In studies of CTA, internal malaise (illness) is induced by exposure to x-rays or injection of pharmacological agents which act on specific sites (e.g. the viscera, area postrema, periaqueductal grey). Gustatory-visceral cues form a strong connection in "bait shyness." Rats more readily associate taste with internal cues, such as illness, than with telereceptive cues, such as vision or audition. Thus, an animal which has previously ingested a poisonous substance typically does not avoid the location or the sight of the bait but, after tasting a sample, does avoid further ingestion of that substance (Garcia & Ervin, 1968). Rotation can also be used as an illness-producing agent. While rotation directly affects the vestibular system, the resulting visceral illness is commonly recognized to be the putative US in producing motion-induced CTA (Braun & McIntosh, 1973; Green & Rachlin, 1973).

Of particular interest to the present study is the concept of exposing animals to a stimulus before it is used as an US for conditioning. Typically, an animal will develop a weaker CTA when it has previous experience with an US than

when that stimulus is a novel US (Kalat, 1974). It has been demonstrated in rats that even a single exposure to pharmacological USs can retard subsequent acquisition of learned taste aversions and that multiple exposures can effectively eliminate the formation of CTA (Berman & Cannon, 1974; Cannon, Berman, Baker & Atkinson, 1975).

Braveman (1975) further investigated the attenuating effects of preconditioning exposure to illness. He exposed animals to pharmacological agents and trained them to avoid a novel-tasting solution with a mechanically-induced illness (rotation) as the US. He gave rats five exposures to one of several toxic chemicals (lithium chloride, amphetamine, or methylscopolamine) before using rotation as an US. Despite a change from pharmacologically-induced to rotationally-induced illness, aversion was attenuated in all groups. Braveman also included a group that received five preconditioning exposures to rotation. This group exhibited a similar attenuation of CTA when rotation was later used as an US. Similar effects when rotation was used as an US were reported by Haroutunian, Riccio and Gans (1976) who also found that five pretraining exposures to rotation greatly reduced the formation of CTA.

The most natural use for rotation is in studies involving motion sickness. Motion-induced illness is often difficult to measure behaviorally or physiologically. Aversion to a novel tasting substance has been proposed as an indicant of the formation of malaise or illness due to rotation (Mitchell, Krusemark & Hafner, 1977; Ossenkopp, 1983). Given that previous exposure to motion, or to motion sickness, apparently can seriously reduce the magnitude of CTA, a confounding effect may occur if experimental animals are used in multiple studies. To maximize cost-effectiveness, animals such as primates are

used frequently over a period of time in a number of research projects. Also, animals are sometimes tested for susceptibility to motion sickness prior to inclusion in an experiment. Thus, if animals in a motion study have previous differential experience with motion, then the resulting CTA may not reflect the true effects of the experimental manipulations.

In the rotation studies by Braveman (1975) and Haroutunian et al. (1976) all rats were exposed to the same number of preconditioning trials so it is not possible to determine the minimum number of exposures to rotation necessary to attenuate CTA. Cannon et al. (1975), using pharmacological agents, found one preconditioning exposure sufficient to weaken a chemically-produced CTA. They also found the degree of attenuation to be positively related to the number of exposures. An investigation into the effect on CTA of various numbers of exposure to rotation with rotation as a subsequent US should yield similar results. That is, animals receiving one preconditioning exposure to rotation should demonstrate a slight weakening of aversion. Animals receiving three exposures should show a greater attenuation of aversion and animals receiving five preconditioning exposures should not develop any significant CTA, much the same as the results obtained by Braveman (1975) and Haroutunian, et al. (1976).

If prior exposure to the US decreases the magnitude of CTA, then this prior exposure should also facilitate extinction. Elkins (1974), using cyclophosphamide as an US, found that six preconditioning cyclophosphamide injections markedly facilitated extinction while three injections resulted in a small acceleration of the extinction rate, and one injection produced no observable effect. Elkins (1973) also investigated the effect of prior exposure to

the CS on resistance to extinction and obtained results that agree with these effects of prior exposure to the US on the strength of conditioning. Generally, preconditioning exposures to a stimulus subsequently used in conditioning produce more rapid extinction, in proportion to the number of exposures.

The purpose of this study, then, was to investigate whether there is a systematic, progressive decrease in the magnitude of motion-induced CTA as the number of exposures to rotation prior to conditioning increases. Such a decrease in magnitude has been shown with pharmacological agents, but has not yet been examined parametrically with rotation. Briefly, the CTA paradigm employed in this study used a saccharin-flavored water solution as the CS and rotation as the US. The rotationally-induced illness was the unconditioned response that, when paired with the saccharin solution (the CS), produced the conditioned response of avoidance of that solution. Of critical importance is the manner in which the dependent variable is measured. It has been noted that the one-bottle test and the two-bottle test are differentially sensitive for detecting conditioning (Spector, Smith & Hollander, 1981). Both tests were therefore employed in this study. The initial magnitude of CTA was measured with a short-term one-bottle test of absolute CS fluid intake. A long-term two-bottle test was employed to measure absolute CS intake over the 10 days following conditioning. The 10-day period was used to evaluate resistance to extinction as a function of the number of preconditioning exposures to rotation.

Method

Subjects

Forty Long-Evans male, naive rats bred at Simonsen Laboratories, Inc. in Gilroy, California were used as subjects. At the start of the experiment, the

weights of the animals ranged from 265 - 330 g. Upon arrival they were individually housed in plastic cages (20 X 24 X 45 cm) with wire mesh on the top. Prior to the start of the experiment rat chow and tap water were available ad lib. The animals were acclimated to the laboratory environment for 7 days. A 12:12 light:dark cycle was used with lights coming on at 4:00 a.m. Preconditioning exposure and testing occurred at approximately the tenth hour of the light cycle. Ten rats were assigned to each of four treatment conditions. Animals in the Control Group (C) received no rotation prior to conditioning, those in the three treatment groups were exposed to rotation 1, 3, or 5 times prior to conditioning (respectfully, groups R1, R3, R5).

Apparatus

The rotation device consisted of an aluminum disk mounted directly on the shaft of a gear reduction box driven by a variable speed motor. The platform of this device was tilted 20° from earth vertical. A Plexiglas holding cage for the rats was bolted onto the aluminum disk which rotated at 150°/s for 30 min. The holding cage contained five tiers of four individual compartments measuring 16 x 15 x 10 cm. The compartments on each level were four squares contained within a larger square, their common corner directly on the axis of rotation. The bottom of each compartment was fitted with wide-grid wire mesh.

The wire mesh covering the home cages was standard heavy-gauge stainless steel cage covering modified to hold two water bottles, one on each side of the cage, with a compartment for food separating the bottles. Glass bottles were used to provide room temperature fluid for the animals to drink. These bottles were fitted with rubber stoppers and stainless steel drinking

spouts which contained ball bearings to limit the amount of seepage. A 0.1% saccharin solution (w/v) was used as the novel taste for conditioning.

Procedure

After the 7-day period of acclimation to the laboratory, the animals were adapted to a restricted drinking schedule by gradually reducing the duration of daily water access from continuous access to 12 h (2 days), then 6 h (2 days), then 2 h (2 days), and finally 10 min followed 1 h later by another 10-min period (2 days). This procedure was to facilitate rapid adjustment to the water deprivation schedule after preconditioning exposure to rotation was completed.

On Day 16, preconditioning sessions began with the animals in group R5 receiving 30 min of rotation. Those animals not rotated, including the controls, were placed in similar Plexiglas holding cages on the device next to the rotating platform for 30 min. This procedure exposed the animals to noise and vibration similar to that occurring during rotation. These sessions were conducted every 4 days for a total of five separate sessions with group R3 being phased into rotation on the third session and group R1 phased in on the last session so that all groups completed the preconditioning exposure regimen on the same day. During this time water was available ad lib.

After the last preconditioning session all animals were returned to the restricted drinking regimen of 10-min access followed 1h later by another 10 min period of access to water. On the 4th day of water deprivation all the animals were presented with the saccharin solution in lieu of the standard water in the first 10-min drinking period. Within 5 min after this drinking period the animals were placed in the holding cages on the rotating platform, rotated for 30 min, and then returned to their home cages for a 2-day recovery period with the

same restricted water regimen. On the 2nd day of recovery the subjects were again offered the saccharin solution for 10 min. The amount consumed was measured by weighing the bottles before and after drinking. One hour after the saccharin was removed, two bottles were introduced into the cage. One bottle contained the saccharin solution, the other contained water. Measurements of drinking preference were taken daily after a period of 21 h. This two-bottle test continued for 10 days with the two bottles alternated between the sides of the cage daily to control for position habit.

Results

Three separate one-way analyses of variance on group weights revealed no reliable differences between groups at the beginning of the study, on the conditioning day, or at the end of the experiment [$F(3,36) \leq 1.82$, $p \geq .25$].

One-Bottle Test

The mean consumption of saccharin solution by the four groups on the conditioning and test days is shown in Figure 1. The average consumption of saccharin by each of the four groups prior to rotation on the conditioning day is shown by open bars. A one-way ANOVA was computed on saccharin consumption by the four groups on this day to determine whether saccharin consumption by the groups differed prior to conditioning. There was no reliable difference among groups [$F(3,36) = 1.97$, $p > .1$] indicating all groups consumed approximately equivalent amounts of saccharin in this initial, or baseline, period.

A second one-way ANOVA was computed on saccharin consumption by the four groups on the test day (shown by hatched bars in Figure 1). This analysis revealed a reliable difference in saccharin consumption on the test day after

Figure 1. Mean amount of 0.1% saccharin solution consumed during 10-min sessions in the one-bottle test. Animals in Group C had no prior exposure to rotation while those in Groups R1, R3, or R5 had 1, 3 or 5 preconditioning exposures, respectively. The open bars represent saccharin intake immediately prior to pairing with rotation. The hatched bars represent saccharin intake 2 days following conditioning.

drinking saccharin was followed by (i.e., paired with) rotation [$F(3,36) = 24.92$, $p < .001$]. The Scheffé Test of pairwise mean differences was used to make post-hoc comparisons to interpret this omnibus effect. This test revealed no reliable difference between Groups C and R1 ($p > .05$). Additional tests indicated that both R3 and R5 consumed more saccharin than Group C and Group R1 (p s $< .001$). There was no difference between Groups R3 and R5 ($p > .05$). Thus, the overall difference between groups in the omnibus ANOVA is due to a similarity in saccharin consumption by Groups C and R1 and the accompanying higher levels of saccharin consumption by Groups R3 and R5.

Effects reflected by this single bottle test were investigated further by examining changes in intake in each group individually using Student's t Test for dependent samples. The average daily intake (and the S. D.) of water on the day prior to conditioning and of saccharin on the conditioning and test days is shown for each group in Table 1. The formation of CTA was reflected by reduced consumption of saccharin on the test day relative to the conditioning day for Groups C [$t(9) = 7.81$, $p < .001$] and R1 [$t(9) = 2.53$, $p < .05$]. In contrast to this effect, Groups R3 and R5 not only failed to demonstrate an aversion but instead increased consumption of saccharin to average levels exceeding that of conditioning day. This difference was reliable for both Groups R3 [$t(9) = 5.41$, $p < .001$] and R5 [$t(9) = 8.02$, $p < .001$].

The relative magnitude of these effects can be seen by comparing saccharin intake to the intake of tap water prior to conditioning. Consumption of saccharin in lesser amounts than tap water on the previous day reflects the commonly observed neophobia seen when a novel fluid is offered in lieu of water. In Groups C and R1, which formed CTA, this effect was enhanced by

Table 1

Fluid Consumption (Grams). Single Bottle. 10 min. Access

Group	Water		Saccharin			
	<u>Pre-conditioning</u>		<u>Conditioning</u>		<u>Single-bottle test</u>	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
C	11.08	1.85	7.06	1.58	2.92	2.69
R1	11.13	1.26	5.76	1.47	3.38	1.87
R3	11.81	2.44	6.43	2.33	10.27	2.99
R5	11.77	1.63	5.11	1.46	9.36	2.08

even less intake of saccharin on the next opportunity (i.e., on the test day). In Groups R3 and R5, which did not demonstrate a CTA, consumption of saccharin approached the daily consumption of water prior to conditioning. In fact, statistical tests of the difference between consumption of saccharin on the test day and consumption of water prior to conditioning indicated no significant difference in Group R3 [$t(9) = 1.75, p > .05$] and only a marginally significant difference in Group R5 [$t(9) = 2.77, p < .05$].

Two-Bottle Test

Saccharin consumption during the 10 days of the two-bottle choice test is shown in Figure 2. The first day with this procedure immediately followed the one-bottle test described above. The general effects reflected in the one-bottle test are also revealed in the data of this day: saccharin consumption is greater for Groups R3 and R5 than it is for Groups R1 or C. Over the 10 days of this test saccharin consumption changed very little for the groups receiving three or five preconditioning exposures to rotation. On the other hand, some evidence for extinction of the CTA is seen in those groups receiving none or one preconditioning exposure to rotation. This is reflected by a gradual, slight increase in saccharin intake over the 10 test days.

A 4 (Groups) X 10 (Days) mixed ANOVA with repeated measures on the second factor was computed to evaluate these effects. This analysis reflected reliable effects for Groups [$F(3,36) = 3.64, p = .02$] confirming that exposure to rotation prior to conditioning reduced the magnitude of CTA, as shown in the one-bottle test. There was also a reliable effect for Days [$F(9,324) = 3.98, p < .001$] indicating a change in saccharin consumption over time for some groups.

Figure 2. Mean saccharin consumption on each of the 10 test days for animals in the control and three prior-rotation groups during the 2-bottle choice test. The Control Group received no prior exposure to rotation. Groups R1, R3, and R5 received 1, 3, or 5 preconditioning exposures to rotation, respectively. Data for each day represent intake of the saccharin solution over a 21-hr period.

The interaction of Groups and Days was expected, but this analysis indicated this effect was not reliable [$F(27, 324) = 1.41, p = .09$].

The apparent parallel between effects in this two-bottle test and the one-bottle data reported above was investigated further by computing a one-way ANOVA on consumption on Day 1. This analysis revealed a reliable overall difference between average consumption by the four groups [$F(3,36) = 6.68, p < .01$]. A Scheffé Test was used to examine differences between individual means. As in the one-bottle test, consumption by Groups C and R1 did not differ ($p > .05$), nor was there a difference between Groups R3 and R5 ($p > .05$). Both Groups R3 and R5 consumed more than Group C (p s $< .05$ and $.01$, respectively), but the mean differences between Groups R3 and R5 and Group R1 did not differ significantly (p s $> .05$). A one-way ANOVA computed on the data for the last day of this test indicated these differences were not present in the data for Day 10 [$F(3,36) = 1.67, p > .1$].

The extinction of CTA was investigated further by computing the simple effects of days for each of the four groups. These analyses indicated there was a reliable increase in consumption over the 10 test days for Groups C [$F(9,324) = 2.47, p < .01$] and R1 [$F(9,324) = 2.95, p < .002$], but no reliable change for Group R5 [$F(3,324) = .90, p > .53$]. The same analysis revealed a significant change in group R3 [$F(3,324) = 1.89, p < .05$]. However, upon examination of Figure 2 it can be seen that saccharin consumption for Group R3 roughly parallels that of Group R5 until the last day when the R3 level drops to near the level of R1. This is likely a chance artifact caused by (a) a blocked drinking spout for animal number 22, preventing it from consuming its normal high amount of saccharin and (b) a slight decrease in saccharin consumption for

several other animals in the group. If Day 10 is eliminated from statistical analysis the simple effect of days on Group R3 is not significant [$F(8,288) = 1.63$, $p > .12$].

Discussion

In both the one and two-bottle tests, groups R3 and R5 demonstrated a significantly higher saccharin consumption when compared to the Control Group. In the one-bottle test both groups C and R1 were similar in CTA and both consumed less saccharin than R3 and R5. On Day 1 of the two-bottle test group R1's saccharin consumption was not significantly different from the Control Group but at the same time not different from groups R3 and R5, indicating a possible effect of preconditioning exposure that did not show up on the one-bottle test. By Day 10 of the two-bottle test saccharin consumption for groups C and R1 had risen to levels approaching that of R3 and R5.

The results of this study support, to a degree, other research describing the attenuating effect of prior exposure to an agent to be used later as an US. Groups having a single or no previous exposure to rotation formed a strong CTA while CTA was attenuated in those groups receiving numerous (three or five) exposures to rotation. Similar strength of CTA between the two strong aversion groups, C and R1, contrasted with the two attenuated aversion groups, R3 and R5. A stepwise progression of attenuation from lowest (C) to highest (R5) similar to the typical dose-response curve formed when pharmacological agents are used as an US was expected. Instead, an apparently dichotomous effect was obtained where the groups either exhibited conditioning or they did not.

Although a progressive effect of preexposure was expected, there is some evidence from prior studies which is consistent with the present results. While Cannon et al. (1975) found one preconditioning exposure to ethanol attenuated conditioning, this exposure preceded conditioning by only 1 day. When a single exposure preceded conditioning by 2 or 4 days, as in this study, there was no difference from the control group (Cannon et al., 1975; Elkins, 1974). Apparently if one exposure is to weaken conditioning, it must occur within 24 h of that conditioning (Best & Domjan, 1979). In apparent contradiction is Braveman's (1975) finding that animals receiving one preconditioning exposure to LiCl 10 days in advance of conditioning demonstrated a significant attenuation of CTA when compared to controls. Best and Domjan (1979) attributed this discrepancy to possible "exteroceptive cues." However, examination of the relative strengths of the preconditioning and conditioning doses may reveal some insight into Braveman's results. His animals were preconditioned with a 3.0 mEq/kg injection of LiCl, a dose capable of producing a very strong aversion (Nachman & Ashe, 1973). Ten days later the animals were conditioned with only a moderate dose of LiCl, 1.5 mEq/kg. It is possible that the animals formed no lasting association with the strong pre-conditioning illness. When conditioned with an US of weaker intensity the salience of the illness was less novel and the resulting association was less meaningful so that the final amount of conditioning was not as strong.

A direct comparison of the relative strengths of physiologically-induced illness and rotationally-induced illness is difficult to make. Parametric observations of LiCl-induced illness have been reported but there are no such studies involving rotation. Rotation speeds ranging from 12 to 198 RPM have

been reported. In addition, the number of revolutions, not speed, has been suggested to be of primary importance to the efficacy of rotation as an US (Green & Rachlin, 1976). The present study utilized an US of moderate strength, 30 min at 150°/s (25 RPM) (Sutton, Fox, & Daunton, 1988). The absence of an attenuating affect of one preconditioning exposure presented 4 days prior to conditioning is consistent with other studies employing the same relative strength US.

The finding of no reliable difference between C and R1 in the one-bottle test should not be taken to mean that one preconditioning exposure had no absolute effect on conditioning. While the effects of one preconditioning exposure did not show up on the 10-min one-bottle test, over the 10-day recovery period saccharin consumption for Group R1 was consistently higher than that for the control group. The implication is that one exposure did have an effect on saccharin preference. This finding supports the argument by Spector et al. (1981) that the use of a single dependent measure is not sufficient to fully describe the effects of the experimental manipulations.

The nearly complete blocking of CTA by five preconditioning exposures was predicted from other studies. What was not expected were the identical results obtained by the group receiving three exposures. In the one-bottle test both groups consumed saccharin in amounts approaching their regular water intake. Over the 10-day two-bottle test saccharin intake was at a consistently high level, showing no signs of increasing which would have been indicative of a recovery from conditioning. A control group in which saccharin was not paired with rotation would have been desirable to measure maximum saccharin intake; however, both groups appeared to be near their maximum intake of

saccharin. A majority of animals in both groups routinely consumed 30 to 40 g of saccharin solution daily which equalled or exceeded their preconditioning daily water intake.

While neither of the conditioned groups had a dramatic increase in saccharin consumption during the recovery period, it should be noted that observations were limited to a 10-day period. Elkins (1973,1974) examined recovery from CTA over 30 and 60-day periods with the greatest increases in saccharin consumption occurring beyond the initial 10-day period. Had this study been extended for a longer period of time it is possible that Groups C and R1 would have increased their saccharin intake to levels near that of R3 and R5. It is also possible that Groups R3 and R5 would have shown some small increase in their saccharin consumption due to extinction in some animals in these groups.

As an index of aversion strength the results of the 10-day recovery period indicate that Groups R3 and R5 shared an equally minimal aversion to the saccharin solution. Previous investigations of preexposure to physiological illness-producing agents have shown that as the number of preexposures increases, the aversion to the CS decreases. In the present study it appears that when rotation serves as the US a ceiling is reached quickly, beyond which continued preexposure has little effect. The exact nature of this rapid attenuation is unclear; however, rotationally-induced illness may be operating on different neural mechanisms than physiological agents such as LiCl (Fox & McKenna, 1988), and as such, the learning parameters may be different for physiological and rotational illness. Traditionally, the CTA paradigm has been investigated using either radiation or chemical USs. In these cases illness is a

result of processes to recover homeostasis following cellular insult from radiation exposure or physiological disruption following chemical exposure. Illness is relatively long-lasting as determined by the metabolic processes which eliminate the toxins. Rotationally-induced illness is mediated via stimulation of the vestibular system. When stimulation ceases, recovery from illness begins. This recovery is likely faster than that from chemical toxicosis in that it involves only a return to previous homeostasis without a need to eliminate toxins. It is unknown how long an animal suffers illness or at what point illness ceases to be a factor in conditioning, but it is possible that a moderate amount of rotation may produce a shorter course of illness than a moderate dose of LiCl.

Another possible factor in the rapid attenuation of CTA is the animals' learning to control the effects of rotation. There is very little an animal can do to lessen pharmacologically-induced illness. It is an unavoidable internal process that must be endured. Rotationally-induced illness is caused by an external event: acceleration forces affecting the vestibular system. Voluntary movement during rotation typically increases stimulation to the vestibular system. During an initial rotation experience an animal tends to explore its environment, shifting its head laterally and vertically, and in the process increasing stimulation to the vestibular system. To minimize the effects of rotation the wisest strategy would be to sit still pointed in the direction of travel. Many animals learn this strategy very quickly. On the conditioning day, Groups R3 and R5, due to prior exposure to rotation, may have sat quietly and "rode out the storm," thus reducing their illness to a minimum.

A third factor in the decrease in CTA is simple habituation. After a period of time the animal adjusts to the environment. What were initially novel stimuli, such as noise, the holding cage and rotation, eventually become familiar and are more or less ignored as irrelevant cues. The animals receiving multiple exposures to illness not only became familiar to the feeling of malaise but learned that illness was not contingent upon any other stimuli. When illness was eventually paired with the saccharin solution, the familiarity with the illness weakened the critical association and the resulting conditioning was attenuated.

One other possibility for the similarity of R3 and R5 is that the tests employed were not sufficiently sensitive to detect a difference in conditioning or that acquired associations were not reflected by performance. Riccio and Haroutunian (1977) demonstrated in a transfer paradigm that associations to external stimuli were not apparent with rotation-induced illness but this prior exposure did become apparent after the same external stimuli were repaired with shock. In the taste aversion paradigm when differential exposure to the US results in CTAs of similar magnitude, it is possible that subtle differences may be detected using second-order conditioning with the CSs. The amount of neophobia attached to the new CS may well reflect the magnitude of aversion associated with a novel taste.

Taken as a whole, the results of this study imply that a single preconditioning exposure to mild rotation has no attenuating effect in a short-term one-bottle test but that attenuation may be reflected over a long-term two-bottle test. Multiple preconditioning exposures apparently produce attenuation quickly as there was no difference in either the short-term or the long-term tests

between groups receiving three or five preconditioning exposures. Rotation as an US may be different than pharmacological USs in that rotation produces a shorter-lasting illness, animals learn to minimize the effects of rotation, and, if rotation is presented prior to conditioning, habituation may reduce the saliency of rotationally-induced illness. As a practical matter, when using animals in research involving rotation it would be wise to use naive animals or at least animals who have all had the same amount of experience with rotation.

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