Journal of Comparative and Physiological Psychology
1982, Vol. 96, No. 5, 775-780

0021-9940/82/9605-0775$00.75

Potentiation of Odor by Taste in Rats: Tests of Some Nonassociative Factors

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The contribution of nonassociative neophobia and sensitization to the potentiation of odor by taste in rats was tested in three experiments. In Experiment 1, neophobia for almond odor (O), saccharin taste (T), and odor-taste compound (OT) cues was tested before and after noncontingent lithium chloride poisoning and compared with conditioned aversions produced by OT–LiCl temporal pairing. The OT compound potentiated unconditioned neophobia, but there was no evidence of poison-enhanced neophobia, disinhibition of neophobia, or sensitization by noncontingent LiCl; temporal pairing produced aversions for the compound and its elements. In Experiment 2, generalization to a novel odor was tested after O–LiCl or compound OT–LiCl pairing. The potentiated odor aversion did not generalize to the novel odor; it was specific to the odor paired with taste and LiCl. In Experiment 3, potentiation of the odor component by a discriminant or nondiscriminant taste component was tested. Potentiation was evident only when a novel discriminant taste was in compound with odor prior to LiCl poisoning. These studies support an associative "indexing" hypothesis of the potentiation effect in rats.

Rats react to strong novel taste with finicky hesitation and sampling which depress consumption. This unconditioned neophobic reaction has the same depressive effect on consumption as a conditioned taste aversion produced by pairing the taste with a toxin. In certain cases, the magnitude of the neophobic response is correlated with the strength of the conditioned aversion (Archer & Sjöden, 1979a, 1979b; Nachman, Rauschenberger, & Ashe, 1977), which has led some investigators to suggest that decrements in consumption following flavor–illness pairing may be due to a nonassociative process such as sensitization, i.e., the enhancement of neophobia for any flavor after poisoning (Bitterman, 1976), or to the prevention or disinhibition of neophobia by poisoning (Mitchell, Scott, & Mitchell, 1977). There is some evidence that poisoning can enhance finicky reactions to strong novel tastes (Domjan, 1977; Miller & Domjan, 1981). There is one study interpreted as showing that poisoning disinhibits the neophobic response to sweet water (Mitchell et al., 1977), although that interpretation is controversial (see *Animal Learning & Behavior*, 1978, *6*, 115–124; 1979, *7*, 562–563).

Recently, we reported several ways in which the odor and taste components of a compound flavor conditioned stimulus (CS) interact during conditioned flavor aversion studies. When a novel odor is presented in compound with a novel taste, the neophobic response is enhanced relative to the neophobia for odor and taste separately (Rusiniak, Hankins, Garcia, & Brett, 1979). If the compound is followed by delayed lithium chloride poisoning, it is much more potent than either component alone, and the aversion for the odor component of the compound is potentiated relative to odor conditioned alone (Rusiniak et al., 1979).

Because odor potentiation depends on

This research was supported by National Institutes of Health Grants NS 11618, HD 05958, and AA 03513. We thank S. W. Kiefer and A. G. Rice for critical comments and assistance, and S. L. Belkin, M. M. Jacobson, and M. J. Smith for media and clerical services.

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pairing with taste and poison (Palmerino, Rusiniak, & Garcia, 1980), we have put forth an "indexing" hypothesis: A specific taste indexes a specific concomitant odor as a food cue and mediates the acquisition of a conditioned aversion for that particular odor (Garcia & Rusiniak, 1980). However, the fact that neophobia for the compound appears proportional to the magnitude of odor potentiation suggests that nonassociative factors may be involved. Rather than potentiating a conditioned odor aversion, taste may prevent habituation of neophobia, or compound conditioning may enhance (sensitize) neophobia for any odor rather than the particular odor paired with poison. Similarly, it is conceivable that the taste component of the compound need not be an associative discriminandum for poison. Nonspecific activation of the gustatory system may simply enhance the salience of and neophobia for concomitant odors to enhance conditioning. The following studies were conducted to evaluate neophobia and sensitization as factors in illness-motivated odor potentiation.

General Method

Subjects

Male Sprague–Dawley rats were housed in single cages in a colony room on a 12:12 hr light/dark cycle. Purina Rat Chow was available freely except during test sessions. Throughout the study, water was limited to a 5-min test session and a 15-min supplement in the home cage later in the day. All animals were habituated to the colony for at least 2 wk prior to the experiment.

Apparatus

Test sessions were conducted in drinkometer boxes described in previous reports (Palmerino et al., 1980; Rusiniak et al., 1979). Briefly, the 30 × 15 × 25 cm Plexiglas boxes had a 1.5 × 3.5 cm oval hole that provided access to a single drinking tube recessed 1–2 cm behind the wall. An "odor disk" that was friction-fitted around the spout held filter paper saturated with the olfactory stimulus. Odorants (.2 ml) could be either placed on the filter paper by a syringe and needle without touching the water spout or mixed in the drinking water. Drinkometer boxes were placed in sound-attenuating chambers in a room separate from the main laboratory; white noise (75 dB re 20 $\mu N/m^2$) in the chambers masked extraneous noise. Electronic drinkometers (Grason-Stadler E4690A-1) connected to the drinking spouts, and standard electromechanical recording equipment, monitored licks, latency to the first lick, and session duration.

Procedure

Each experiment began with a habituation phase (10–14 days) when rats were trained to drink unflavored distilled water during 5-min test sessions in the apparatus. When consumption was stable, flavor cues and poisoning were given; extinction tests with the odor and/or taste component followed. Poison-paired flavor cues were given every third day. Saccharin water (.1% or .05%) served as the taste stimulus (T); almond extract or vanilla extract (Schilling) either saturated on the odor disk (.2 ml) or mixed in the drinking water (2%, v/v) served as the odor (O) stimulus. Stimuli could be presented separately or in compound (OT). Poison treatment consisted of intragastric (ig) delivery of .15 M LiCl (190 or 127 mg/kg) from an infant feeding tube (Pharmaseal K-31).

For statistical analysis, nonparametric Wilcoxon ranks (R) and signed-rank tests (signed R) were used for between- and within-groups comparisons, respectively (Langley, 1970, pp. 166–189). For each rat, consumption suppression ratios (A/B) were formed by using flavor trial lick scores (A) and preexperimental water baseline (B); baseline was the lick average from the last 2 days of habituation. Baselines averaged 1,000–1,300 licks and never differed among groups or shifted over the experiment.

Experiment 1:

Neophobia Before and After Poison

The first study was designed to test for unconditioned neophobia and poison-enhanced neophobia evoked by scented water (O), sweet water (T), and scented-sweet water (OT) and to compare those effects with conditioned aversions produced by temporal pairing with poison.

Procedure

Groups of rats habituated to drinking during daily 5-min test sessions received a single LiCl (190 mg/kg) poisoning trial and then were assigned randomly to one of three groups for extinction tests with almond alone (O), saccharin alone (T), or the almond-saccharin compound (OT). The associative group (Paired) received a 2% almond-scented .1% saccharin solution 30 min prior to LiCl and three extinction tests (OT–O, n = 12; OT–T, n = 10; OT–T, n = 10). One nonassociative group (Unpaired) received the OT compound the day before receiving water, which was followed by 30-min-delayed LiCl and three extinction tests (OT/O, OT/T, and OT/OT; n = 10 each). Another nonassociative group (Novel) received water 30 min prior to LiCl and the test flavors for the first time during extinction (W–O, W–T, W–OT; n = 10 each).

Results and Discussion

Mean consumption ratios are shown in Figure 1. The Paired associative groups (left panel) showed strong neophobia for the OT compound during the acquisition trial prior to lithium treatment, as each group reduced consumption relative to water baseline (signed Rs ≤ 3, ps $\leq .01$). On the first extinction test, only Group OT–OT decreased consumption reliably below the acquisition-trial neophobia level (signed $R = 0$, $p < .01$), but relative to water baseline all three groups showed a reliable decrement (signed Rs < 2, ps $< .01$). The Unpaired nonassociative groups (middle panel) also exhibited neophobia for the OT compound before poisoning (signed Rs < 6, ps $< .01$) but drank the test fluids at water baseline after unpaired lithium treatment. Therefore, it appeared that temporal pairing of the flavor with poison produced an associative aversion for the compound and both of its components. Unpaired lithium treatment did not produce an aversion for any flavor by disinhibiting the neophobic response.

The Novel nonassociative groups (right panel), which received the test fluids for the first time after unpaired lithium treatment, also showed neophobia for the OT compound on the first presentation (signed $R = 0$, $p < .01$); the components O and T did not evoke any reduction in consumption. Group W–OT, drinking the OT compound after unpaired lithium, did not differ from pooled OT groups (Paired and Unpaired) drinking OT prior to lithium ($R = 252$, $p > .05$), which indicates that there was no poison-enhanced neophobia. Also note that virtually no suppression was evident in any nonassociative group on the second exposure to a flavor, which indicates that neophobia dissipated after a single flavor trial.

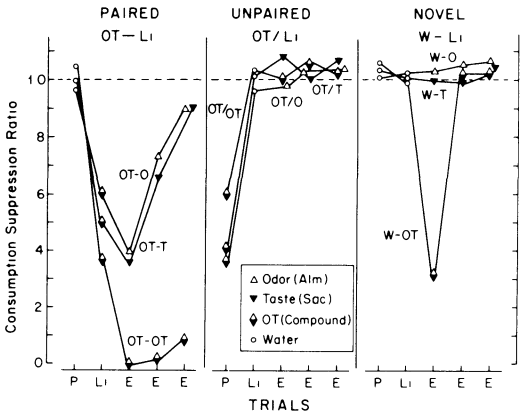

Figure 1. Consumption of scented (O), tasty (T), and scented, tasty (OT) water before (P) and after lithium poisoning. (Flavors were paired with the OT compound to produce associative aversions [left], unpaired with flavors to test for disinhibition of neophobia [center], or presented for the first time after poisoning to test for poison-enhanced neophobia [right]. E = extinction.)

Experiment 2:

Odor Sensitization and Specificity

The second study tested whether taste potentiates an aversion for the specific food odor used in acquisition or whether it causes a generalized avoidance of other food odors as well.

Procedure

After habituation to drinking water during 5-min trials in the apparatus, two groups received three acquisition trials when a flavor cue was followed 30 min later by LiCl treatment (190 mg/kg). Group OT ($n = 12$) was a potentiation group that received .2 ml of a flavor extract on the odor disk, together with .1% saccharin in the spout; Group O ($n = 12$) was an odor–only control that received flavor extract on the odor disk and distilled water in the spout. Half of each group received almond extract as the lithium-paired odor, and half received vanilla extract. All rats then received three odor–only extinction tests. On the first and third extinction trial, the lithium-paired odor extract was presented on the odor disk with distilled water in the spout. On the second extinction test, all groups were tested with a novel odor. Groups trained with almond were tested with vanilla extract on the odor disk and distilled water in the spout; conversely, groups trained on vanilla were tested with almond. There were no differences between almond and vanilla as cues in Group O and OT, so the subgroups were pooled.

Results and Discussion

The results (shown in Figure 2) indicate that Group O did not reduce consumption below the neophobia of the first acquisition or water baseline on any trial. Odor alone was a weak cue for delayed LiCl, and repeated poisoning did not enhance neophobia for a novel odor. In contrast, Group OT, the potentiation group, progressively reduced consumption during compound acquisition. Relative to both water baseline and first-trial odor neophobia (Group O, first LiCl trial),

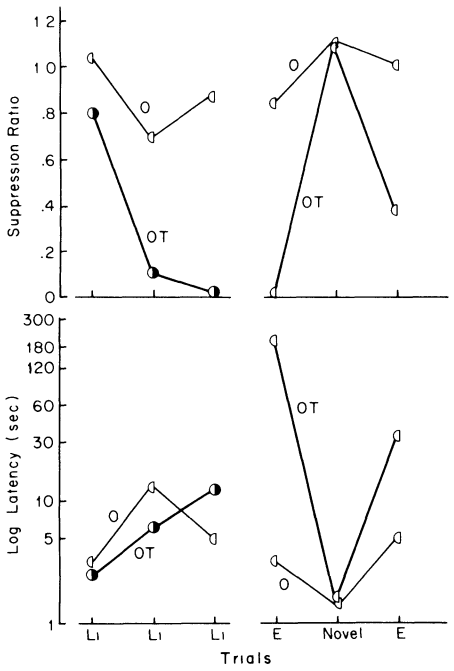

Figure 2. Consumption in the presence of lithium-paired and novel odors (right) after single CS (O) or compound conditioning (OT; left). (E = extinction, mean consumption ratios.)

Group OT continued to suppress drinking on the first and third extinction tests with the lithium-paired odor ($Rs < 3$, signed $Rs < 3$; $ps < .05$). Consumption on the second extinction test with the novel odor was at water baseline and at least equivalent to the unconditioned odor neophobia, an effect indicating that the conditioned odor aversion did not generalize to the novel odor. Note that the consumption effects were also evident in the latency measure, a result indicating an odor aversion that inhibited the first lick at the spout. The present results clearly suggest that taste potentiates an aversion of the specific odor paired with taste and poison.

Experiment 3:

Taste Discrimination in Potentiation

In the third study, we varied the predictive, discriminate role of the taste component of the OT compound to test whether generalized activation of the taste system would be sufficient to potentiate odor aversions or whether taste also must be predictive of illness. If nonassociative taste activation is sufficient, then any type of taste system activity should potentiate odor aversions. If taste must be part of the discriminandum, then odor potentiation should be related to the associative properties of the taste component preceding poison.

Procedure

After habituation to drinking water during 5-min test sessions in the apparatus (14 days), four groups of rats were given two-odor discrimination training. On lithium acquisition trials, almond extract (O_1, poison odor) was presented on the odor disk and followed 30 min later by 127 mg/kg LiCl treatment. There were seven almond presentations during training, one every third day; the first was an unpoisoned pretest, and the next six were lithium acquisition trials. On the last seven habituation trials before the first almond trial and on all intervening trials thereafter, vanilla extract (O_2, safe odor) was presented on the odor disk without poison treatment. Different groups had saccharin solution (T) presented in compound with the odors as follows: Group O_1–O_2 ($n = 8$) was an odor–only reference group; distilled water was in the spout on both almond–poison and vanilla–safe trials. Group O_1T–O_2T ($n = 8$) received OT compound lithium acquisition with an unpredictive, nondifferential taste component; .05% saccharin was in the spout on both almond–poison and vanilla–safe trials. Group O_1–O_2T ($n = 6$) received a taste difference, but the potentiating taste was added to the safe vanilla odor, and the poison-paired almond odor was presented with familiar distilled water; .05% saccharin was in the spout on vanilla trials, and distilled water was in the spout on almond trials. Group O_1T–O_2 ($n = 6$) was the potentiation reference group in which a predictive taste was paired with odor and poison; .05% saccharin was presented in compound with the almond odor prior to poison, and distilled water was in the spout on safe vanilla trials. Two days after the last acquisition trial, all four groups were given an odor–only extinction test with almond on the disk and distilled water in the spout. Note that flavored fluids were presented on all 5-min test trials; however, unflavored distilled water continued as the daily home cage water supplement.

Results and Discussion

The consumption data are shown in Figure 3. Despite repeated trial discrimination training, Group O_1–O_2 never reduced consumption below baseline, an effect indicating that distinctive odors marking both poison and safe fluids were ineffective cues for delayed lithium poisoning in the absence of a potentiating taste. In contrast, when a novel

saccharin taste marked the poison-paired odor, Group O_1T–O_2 showed potentiation, reducing consumption below safe flavor baseline on all trials after the first lithium acquisition. This group showed a clear discrimination between poison and safe flavors which was maintained on the extinction test with almond odor alone (signed Rs = 0, ps = .01). The remaining groups exhibited weak discriminations. When saccharin marked only the safe odor for Group O_1–O_2T, consumption was below baseline on the fourth lithium acquisition trial (signed $R = 0, p < .05$), but only four of six animals continued to drink less almond than vanilla on the remaining acquisition and test trials. Similarly, when a constant saccharin taste marked both poison and safe odors, Group O_1T–O_2T developed a weak discrimination, as consumption was below baseline on the odor extinction test (signed $R = 3, p < .05$). However, between-groups comparisons on the odor-alone extinction test indicated that neither of these groups differed from the O_1–O_2 control (Rs = 32 and 52, ps > .10); only Group O_1T–O_2 exhibited a reliable potentiated odor aversion ($R = 22$, $p < .01$). These results indicated that taste must be part of the poison discriminandum. Neither the absence of a safe taste nor the presence of a familiar taste was an effective potentiating agent.

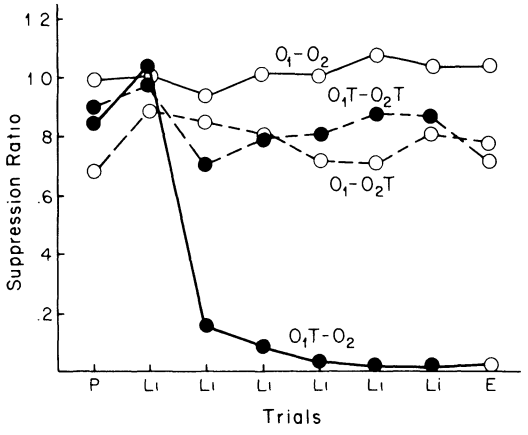

Figure 3. Acquisition of a discrimination between two odors when one (O_1) was paired with poison and another (O_2) was safe. (Tastes [T] were added to one, both, or neither odor during LiCl acquisition to test for potentiation efficacy, indicated on the odor-only extinction test [E], mean consumption ratios.)

General Discussion

The present results, together with previous findings, indicate that the potentiation of odor by taste during toxiphobia studies cannot be attributed solely to nonspecific, nonassociative processes such as differential neophobia or disinhibition of neophobia. Some specific, associative process is more likely to be involved, as postulated in the indexing hypothesis espoused by Garcia and Rusiniak (1980). As shown in Experiment 1, noncontingent lithium poisoning neither enhanced initial neophobia nor reinstated neophobia for previously consumed scented, tasty, or compound flavored solutions. Temporal pairing of the compound flavor with poisoning was necessary to induce an aversion for the compound and its components. This finding is consistent with our previous report that the magnitude of the potentiation effect depends on the CS–US (unconditioned stimulus) delay (Palmerino et al., 1980). Further, as shown in Experiment 2, compound conditioning produced an aversion for the specific odor paired with taste and lithium toxicosis, not a generalized rejection of other novel odors. We have also reported little cross-modal generalization between taste and odor conditioned as single CSs (Rusiniak et al., 1979). Finally, as shown in Experiment 3, the discriminative, associative properties of the taste component of the compound preceding poison determine potentiation. Nondiscriminate tastes or taste cues correlated with safe odors, weak cues for poison themselves, were not effective potentiating agents. A novel, distinctive taste correlated with odor and poison was necessary to produce strong potentiation. That is consistent with our prior finding that potentiation was directly related to the intensity of the taste component on the acquisition trial (Rusiniak et al., 1979). We should also note that neophobia and sensitization were probably not problems in our previous work because all of our studies employed stimuli and procedures similar to those of the present report.

There are other nonassociative factors besides neophobia and sensitization which were not tested. Galef and Osborne (1978) suggested that a novel taste may focus at-

tention on the other food cues. Rats drinking scented, tasty fluids and pigeons drinking colored, tasty fluids for the first time have been observed to show a pattern of sampling broken by intense sniffing and gazing at the fluid. Increased attention toward the CS may facilitate odor conditioning in rats and color conditioning in pigeons. However, some of our other work (Rusiniak, Palmerino, Rice, Forthman, & Garcia, 1982; Rusiniak, Lopez, & Rice, Note 1) is not consistent with a purely attentional explanation, as taste does not potentiate odor–shock conditioning. Rather, the data are more consistent with the notion that the taste of a food mediates an associative conditioned aversion for concomitant odors following illness, as postulated in the "indexing" hypothesis (Garcia & Rusiniak, 1980).

Reference Note

1. Rusiniak, K. W., Lopez, S. C. & Rice, A. G. *Odor and taste: Potentiation with illness, overshadowing with shock.* Paper presented at the meeting of the Western Psychological Association, San Diego, California, April 1979.

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Received October 26, 1981 ■