Orosensory factors in the ingestion of corn oil/sucrose mixtures by the rat

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Abstract

The experiments reported here were designed to study the orosensory factors contributing to the ingestion of sucrose/corn oil mixtures. When a flavor aversion was conditioned to the sucrose/corn oil mixture, the subsequent aversion to the mixture strongly generalized to the corn oil but very little to the sucrose. Rats conditioned with corn oil show a more profound aversion to the sucrose/corn oil mixture than rats conditioned with sucrose, indicating that the salient feature of the sucrose/corn oil mixture is the oil. Aversion to the sucrose/corn oil mixture does not generalize to a sucrose/mineral oil mixture, giving evidence that the textural aspects of the oil do not play a major role in its perception. This flavor aversion to the mixture is further illustrated in very short-term tests where postigestive factors are minimized, indicating a role for the gustatory system in the detection of the sucrose/corn oil mixture. Preliminary experiments are reported where conditioning tests were run with mixtures of sucrose and linoleic acid, one of the fatty acids that is possibly derived from a breakdown of the corn oil in the oral cavity by lingual lipase from von Ebner's Gland. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

Fat intake results from both postigestive and orosensory controls. The orosensory factors would include texture, taste, and olfaction. Only recently has attention been directed to the relative importance of these factors in the control of fat intake in humans [28,29] and in rodent models [1,2,7,8,14,15,17,21–25,32].

It is well known that postigestive factors influence fat intake. In most of the studies using rats as subjects and corn oil as the fat, it has been shown that the baseline diet, the deprivation state, and the past experience with corn oil will influence the subsequent intake of fat. For example, Reed et al. [25] showed that nondeprived sham-feeding rats initially consume more corn oil in 30-min tests if they have been maintained on a high-fat diet in contrast to a high carbohydrate diet. Although this difference in intake disappeared after a few days of testing, it supports the idea that there are definite postabsorptive controls when fat is consumed in liquid form. Ackoff and colleagues [2] reported that intake of either corn or mineral oil is enhanced with food deprivation. They also showed that deprived rats prefer corn oil to mineral oil, but conclude that these results come from learning through postigestive factors. They concluded that rats are attracted to the flavor of emulsified oils from a very early age. Greenberg and Smith [14,15] reported that there was a marked increase in consumption of either corn oil or mineral oil in sham-feeding tests that were conducted after 18 h of food deprivation. If the rats were not food deprived before the sham tests, they consumed considerably less of the oils during the 30-min tests.

Among the orosensory factors influencing oil intake, the importance of the texture of the oil has received only minimal attention. If texture (mediated by the trigeminal nerve) plays a major role in intake, comparisons of the ingestion of mineral (nonnutritive) and corn oil (nutritive) would be of interest. Greenberg and Smith [14,15] compared the ingestion of corn oil and mineral oil in 30-min sham-feeding tests. When the rats were food deprived for 18 h before the initiation of the test, both the mineral oil and the corn oil groups consumed about 25 mL during the 30-min test. One could conclude from this that the texture, not the taste, smell, or nutritive value played a major role. However, when the corn oil group was switched to mineral oil and the mineral oil group was switched to corn oil, the latter group consumed about twice as much corn oil as the other group consumed mineral oil. Furthermore, when the rats from either group had access to both bottles of oil during a sham feeding test, they drank mostly corn oil. They concluded that both oils have orosensory stimulating properties that are sufficient to result in vigorous sham feeding, and that in this case the conclusion would be that this feeding would not likely be the result of any postabsorptive metabolic con-
sequence. Finally, they concluded that because there was such a strong preference for corn oil over the mineral oil the controls for intake and for preference were different. They proposed tactile and olfactory receptors as the possible neurological substrates for these intake behaviors.

Larue [16] used a conditioned flavor aversion design to study the orosensory similarities of three kids of fat (butter, margarine, and lard) and to compare them with a nonnutritive mineral, Vaseline. She found that when using one of these fats mixed with a powdered diet as the CS, which was paired with a LiCl injection, there was not only an aversion to that particular fat, but the aversion generalized to the other fats. However, it did not generalize well to the Vaseline. Ramirez [21] also studied the rat’s orosensory qualities in discriminating different kinds of oils. He showed that when the rat was conditioned to one kind of oil, discrimination among oils of various viscosities was possible. He concluded that the lubricating qualities of the oil provided a cue to the sensory discrimination.

More recently, Gilbertson and colleagues [9–13] have proposed that rats taste fatty acids that represent potential chemical cues contained in dietary fat. They have shown that certain fatty acids delayed rectifying K+ (DRK) channels in isolated rat taste receptor cells that could lead to increases in cellular activity. They propose that these increases in cellular activity represent a taste cue for dietary fat. Electrophysiological evidence shows that the degree to which these fatty acids inhibit DRK channels differs significantly in fat-prefering and fat-avoiding strains of rats. If the rat is having a gustatory experience from ingesting fats, they assume that the rat has enough lingual lipase (probably from Von Ebner’s gland) to start the process of breaking down the fats into “tasteable” fatty acids.

In contrast, Schiffman et al. [29] reported finding no lingual lipase in the human mouth after subjects had either chewed or held in their mouths fat or control substances for a 4-min period. They report that lingual lipase is present in human infants, but it is controversial as to whether these enzymes affect adult human taste of fats.

Ackroff and Sclafani [1] measured intake of high fat foods associated with the ingestion of orlistat, an inhibitor of pancreatic lipase. In short-term two-jar ingestion tests they found that the rats needed three daily test sessions before they ate less of the orlistat adulterated fat food compared to the plain fat food. They concluded that rats could detect a flavor difference between the orlistat-adulterated fat and plain fat, but that the postigestive actions of the drug were primarily responsible for the reduced intake of the orlistat-adulterated fat.

The only direct evidence that olfaction plays no role in the ingestion of fats is presented in the study by Larue [16]. She studied the generalization across three kinds of fat (butter, margarine, and lard) and compared them with a nonnutritive mineral, Vaseline. When she made rats anosmic, they maintained their ability to discriminate the fats from the Vaseline. Her conclusion was that both the greasy texture and the taste may contribute to this generalization.

In many of the studies cited above, the corn oil is blended with water using an emulsifier such as Tween-80. In two-bottle reference tests in our laboratory we have found that rats do not prefer or reject Tween-80 when it is paired with water, so it is assumed that it plays no role in ingestion of the corn oil emulsions. The only study measuring a corn oil concentration preference curve was conducted by Greenberg and Smith [14,15]. They showed that the amount of corn oil ingested depended on the concentration of the oil when it was blended with water. The largest intake occurred when the concentration was about 25%, but the rats still took in about 30 mL when pure corn oil was used. This inverted U-shape intake curve is not unlike that seen with varying concentrations of sucrose solutions [27].

The relative roles of orosensory and postigestive factors of sweet/fat mixtures in rats have received even less attention than that of fat alone [8,17,22,32,34]. Understanding the orosensory factors in the ingestion of sweet/fat mixtures is important because in the human diet fat is most often consumed in conjunction with sweeteners [4–6], and is very important in weight control.

Careful observations were made by Lucas and Sclafani [17] comparing the intake and weight gain of three groups of rats given a sweet/fat mixture (35% corn oil blended with 8% sucrose solution), a sweet solution (8% sucrose), or a fat mixture (35% corn oil blended with water). The intake and weight gains of these three groups of rats were compared with a control group fed only Purina Lab Chow. All of the groups had access to a water bottle and to the lab chow. The mixture of sucrose and corn oil resulted in enhanced caloric intake and to a significant increase in body weight. To determine if the increased intake of the sucrose/fat mixture was the result of the sweetness, the group that had received only the corn oil mixture was subsequently tested with a 0.2% sodium saccharin solution rather than with water mixed with the corn oil. The intake of this sweetened/fat solution over the fat alone was increased immediately. Further evidence that the palatability of the sweetened/fat solutions played a major role in the enhanced intake was shown in two-bottle preference tests between the oil alone and the oil plus sweetener. It was demonstrated that saccharin/oil was preferred over oil, that sucrose/oil was preferred over oil, and that sucrose/oil was preferred over saccharin/oil mixtures. Because it is well known that sucrose is preferred over saccharin [3,35], it seems likely that the increase in intake of these two oil/sweet solutions is the result of taste.

Reed and Friedman [22] tested rats in 30-min tests with mixtures of various concentrations of sucrose and corn oil. In a Latin Square design with ranges of sucrose concentration from 0 to 40% and ranges of corn oil concentration from 0 to 20%, they showed that intake of these mixtures increased with increases in both the sucrose and the corn oil, not unlike what Warwick and Schiffman [34] reported for normal weight adult humans. For the rats, Reed and Friedman showed that when the fat/sweet mixture was 20% corn oil and 40% sucrose, their nondeprived rats consumed
nearly 15 mL of this mixture in 30 min. This large intake in such a short time period should give evidence that orosenry factors play a role in the ingestion of the sugar/fat mixtures. However, half of their rats had been maintained in their home cages on a high-fat diet and the other half on a high-carbohydrate diet. In these 30-min tests, the rats on the high-fat diet drank more of the sucrose/corn oil mixtures than those on the high-carbohydrate diet. The rats that were maintained on this carbohydrate diet drank only about 10 mL of the 20% corn oil and 40% sucrose mixture compared to the 15-mL consumption of the rats on the high-fat diet. Reed and Friedman concluded that although the rats could discriminate between the different flavors on the basis of orosensory factors regardless of the maintenance diet, flavor alone could not account for the increased intake of the corn oil/sucrose mixture by the rats that were maintained on the high-fat diet.

Orosensory factors influencing the ingestion of sweet/fat mixtures involves textural, taste, and possibly olfactory cues [26] in addition to the postdigestive factors. Using a conditioned flavor aversion design, the present set of experiments was designed to study the orosensory factors that contribute to the ingestion of sweet/fat mixtures. The experiments used sucrose as the sweetener and corn oil as the fat. Attempts were made to isolate the most salient component of this mixture that has been shown to result in marked increases in caloric intake and weight gain [17,22].

2. Materials and methods

2.1. General procedure

The general procedure used was to condition a flavor aversion to a sucrose/corn oil mixture and to test for flavor generalization to the components of the mixture. This procedure has been widely used to determine similarities of tastes by the rat [8,16,18–20,30,31]. The subjects for the experiments were both male and female albino rats from Charles River Laboratories. Previous unpublished experiments in this laboratory have shown that there are no differences between the sexes in conditioning aversions to corn oil. The weight and sex of the rats will be described for each experiment. They were individually housed in Plexiglass cages with wood shavings as bedding. The temperature controlled experimental room was on a 12:12 light:dark schedule, with the lights coming on at 0700 h. The rats had continuous access to Purina Chow Pellets (#8001). To ensure that the rats consumed some of the conditioned stimulus fluid on the conditioning day, the rats were placed on a schedule restricting access to water to limited time periods. Seven days before conditioning the water bottles were removed from the home cages for 24 h. For 2 consecutive days following this deprivation period they received 1 h of access to a water bottle in their home cage at 1000 h. On the next 4 days their water access period was reduced to 30, 20, 10, and 10 min, respectively. Conditioning day followed, during which the rats had access to the conditioned stimulus fluid. The duration of the CS is described in each experiment. Immediately following this CS drinking period, the experimental rats received an i.p. injection of 0.6 M LiCl at a dose of 0.005 mL per gram of body weight. This dose was described by Nachman and Ash [18] as an adequate concentration and dose level for producing a reliable flavor aversion with only one pairing. The control rats received an equal volume of 0.15 M NaCl. On the subsequent test day the rats were given a two-bottle preference test with the appropriate solutions. The durations of the preference tests are described in each of the experimental procedures. During the preconditioning deprivation and throughout the postconditioning testing period the rats received a 30-min water supplement later in the afternoon in order to ensure adequate hydration. Postconditioning flavor aversion is described with preference scores (CS intake/CS intake + vehicle intake). Analyses were performed with appropriate ANOVAs or with one-tailed t-tests.

2.2. Calibration of the phase separation of the sucrose from the corn oil

For most of the experiments, various intake preference tests were administered when corn oil had been mixed with sucrose or water. The corn oil/sucrose solution was made by mixing 85.6 g of sucrose in 840 mL of deionized water. This sucrose solution was blended with 160 mL of corn oil (Mazola Brand). Five milliliters of Tween-80 was added to the mixture, and the ingredients were mixed in an industrial blender for 2 min. Diluted corn oil was made in the same manner except that the sucrose was deleted. Separation of the oil from the water (or sucrose solution) occurs fairly rapidly, resulting in a reduction in the proportion of corn oil in the emulsion over the time that the rat would be sampling. Because the longest preference test reported in these experiments lasted for 90-min, the phase separation for that period was calculated in the following manner: A regular 300-mL glass water bottle with a stainless steel spout tube was modified with a stopcock inserted in a hole on the side of the bottle near the bottom. The bottle was filled with the water/corn oil mixture, inverted as it would be on the rat's cage, and attached to a ring stand. At any interval following the blending of the emulsion the stopcock could be opened and a sample of the fluid drained off. An effort was made at these time intervals to remove the amount that the average rat would have consumed during that time period. One-milliliter aliquot samples of the emulsion taken at various times after blending were weighed and allowed to dry for 48 h. This procedure allowed for quantifying the percent of corn oil that was left in the emulsion at that particular time. The quantification curve is shown in Fig. 1. It can be seen that a rat would be getting a 16% corn oil solution soon after the time of mixing, but a 10% solution at the end of a 90-min preference test. It was observed that most of the drinking in the preference tests for the water deprived rats was done
within the first 15 min of the test, reducing the percentage of corn oil in the emulsion by only about 2%.

3. Experiment 1

3.1. Subjects

Sixteen male rats from Charles River ranging in weight from 359 to 444 g were used as subjects.

3.2. Procedure

After the accommodation period described above, the rats received a mixture of sucrose and corn oil as the conditioned stimulus. After 10 min of access to this sweetened fat emulsion, eight of the rats were given the LiCl injection and eight were injected with isotonic saline at the doses previously described. The rats were returned to their home cages and remained on water deprivation for the next 23 h. On the next day the rats from both groups were given a 90-min two-bottle preference test between the sucrose/corn oil mixture and water. On the second testing day the rats received the 90-min two-bottle test between 0.25 M sucrose and water. On the third testing day the rats received a 16% corn oil mixture versus water. This latter mixture was made by adding 840 mL of water to 160 mL of corn oil, blended with 5 mL of Tween-80 as described above. On the fourth day of testing the rats received the 90-min two-bottle test between the 0.25 M sucrose and the 16% corn oil solution. Daily preference scores for each of these mixtures over water were calculated by (mixture consumed)/(mixture + water consumed) for the first 3 days of testing.

3.3. Results

The results of the first 3 days of preference testing can be seen in Fig. 2. From the upper panel it can be seen that there is a sixfold decrease in preference for the sucrose/corn oil mixture when comparing the LiCl injected rats with the saline injected controls, \( t(14) = 6.61, p < 0.001 \). The aversion to sucrose seen in the second panel is significant, \( t(14) = 2.83, p < 0.01 \), but the magnitude of the aversion is only a 1.5-fold decrease. In the third panel it can be seen that there is an eightfold decrease in preference in the LiCl injected group, \( t(14) = 6.47, p < 0.001 \). On the fourth test day when the choice was between sucrose and corn oil, the LiCl group avoided the corn oil, \( t(8) = 13.2, p < 0.001 \). The saline injected rats drank both sucrose and corn oil, but significantly more sucrose than corn oil, \( t(8) = 4.86, p < 0.001 \). The difference in corn oil intake between the two groups was significant, with the saline injected rats drinking six times more corn oil. The LiCl group drank slightly more sucrose than the saline injected group, \( t(8) = 1.79, p < 0.05 \). The preference for fat over sucrose can be seen in the lower panel of Fig. 2. In other tests [32], when pairing sucrose with corn oil, some of the saline injected rats sampled the corn oil and the sucrose in close temporal proximity in a manner similar to the “cocktail mixing” of glucose and saccharin [33].

3.4. Discussion

Because the conditioned aversion to the corn oil/sucrose mixture generalized more to the corn oil than to the sucrose, it can be concluded that the salient orosensory feature of the mixture is the oil. To ensure that the sequence of the preference testing had no effect, the experiment was repeated with 16 different male rats (8 in NaCl and 8 in LiCl groups) where the order of the tests across days was reversed. The results were the same as those previously described. There were no statistically significant differences between the preference tests for each of the trials when the order of presentation was reversed.

The tentative conclusion from these experiments is that the salient feature of the sucrose/corn oil mixture is the oil. It is possible that the stronger aversion to the corn oil after conditioning with the mixture is because it is easier to condition a flavor aversion to corn oil than to sucrose. There is some evidence that less preferred flavors are more effective targets of aversion conditioning. Also, these findings have not ruled out any possible role of olfaction and a taste–odor potentiation. It is known that rats can discriminate sucrose solutions from water by olfaction, but it is likely that the odor of the corn oil is even more potent. When given a mixture of glucose and saccharin followed by an exposure to ionizing radiation, rats have been shown to develop a much stronger aversion to the saccharin than to the glucose [31,33].

The purpose of the second experiment was to compare the magnitude of an aversion to sucrose with an aversion to corn oil in separate groups of rats.

![PERCENT CORN OIL IN MIXTURE](image)

Fig. 1. The curve shows the phase separation of corn oil from either deionized water or a sucrose solution. One hundred and sixty parts of corn oil and 840 parts of water (or sucrose solution) are blended with the detergent, Tween-80 into an emulsion. At time zero this is a 16% solution of corn oil, but the oil and water gradually separate over time. The percent of oil remaining in the useable part of the rat’s fluid container is plotted over the first 1.5 h after blending.
4.3. Results

There were no differences between the preference scores for the males and females; therefore, the data for the two sexes were combined for the analysis. The preference scores for the aversion to sucrose or the aversions to corn oil are seen in Fig. 3. Here, it can be seen that rats conditioned with 0.25 M sucrose developed a profound aversion to this sweetened solution, \( t(15) = 15.59, p < 0.001 \), and the rats conditioned with the corn oil developed a strong aversion to that emulsion, \( t(18) = 6.19, p < 0.001 \). Neither preference scores for sucrose or for corn oil in the NaCl injected rats or the aversion scores for sucrose and for corn oil in the LiCl injected rats were different (\( t \)-values of 1.49 and 0.74).

4.4. Discussion

These data show that the results of Experiment 1 cannot be accounted for by the hypothesis that it is merely easier to get an aversion to corn oil than it is to sucrose.

5. Experiment 3

It had been shown in Experiment 1 that rats that received the sucrose + corn oil mixture as a CS developed a strong preference.

**Fig. 3.** The rats received either a sucrose solution or a corn oil mixture as the CS followed by either a NaCl or a LiCl injection. Aversion to the sucrose solution (left two bars) and the coin oil mixture (right two bars) are illustrated.
aversion to the mixture and to the corn oil and a lesser aversion to the sucrose. It was concluded that the salient sensory feature of the mixture was the corn oil. If this is the case, one could hypothesize that the reverse would be true, i.e., rats that were given corn oil as the CS would show a stronger aversion to the sucrose + corn oil mixture than would rats that had received sucrose as the CS. The purpose of this experiment was to test this hypothesis.

5.1. Subjects

Twelve male rats from the sucrose group and 12 males from the corn oil group in Experiment 2 that had already been conditioned to either sucrose or corn oil were used as the subjects in this experiment. Eight rats from each of the sucrose and corn oil groups that had been conditioned with LiCl injections and four rats from each of the saline control groups composed the 24 subjects.

5.2. Procedure

These previously conditioned rats and their saline-injected controls were given daily 90-min two-bottle preference tests between the sucrose + corn oil mixture and water for 5 days.

5.3. Results

Because there were no differences between the two control groups, they were combined to make one group of eight rats, $F(1, 6) = 0.08, p > 0.05$. The mean preference scores for the sucrose/corn oil, along with the saline injected controls can be seen in Fig. 4. As can be seen, the group that was conditioned with corn oil exhibited a strong aversion to the sucrose/fat mixture across all five days of testing. In contrast, the group that was conditioned with sucrose exhibited a mild aversion to the mixture that was completely extinguished in 5 days. A repeated sampling of the same subjects analysis of variance across the five testing days yielded a significant difference between the three groups, $F(2, 21) = 28.39, p < 0.001$, between the 5 testing days, $F(4, 84) = 20.13, p < 0.001$, and a significant groups × trials interaction, $F(8, 84) = 5.17, p < 0.001$. Subsequent tests showed that there was significant difference between the three groups except on the fifth day of testing when there was no difference between the sucrose and the sham groups, but both of those groups were different from the corn oil group.

5.4. Discussion

These results show that the generalization from the corn oil CS to the corn oil/sucrose mixture was very strong. The generalization from the sucrose CS to the corn oil/sucrose mixture was less strong, and got progressively weaker over the 5 days of testing. These data add to the mounting evidence that the salient feature of the corn oil/sucrose mixture is the corn oil. However, the question regarding the relative roles of texture, olfaction, and taste of the corn oil remains.

Fig. 4. The rats received taste aversion conditioning with sucrose paired with LiCl injections (diamonds) or similar conditioning where corn oil was paired with the LiCl injection (circles). Control rats (squares) received a saline injection. The preference scores for a corn oil/sucrose mixture over 5 days of testing are plotted.

6. Experiment 4

The first three experiments gave evidence that the corn oil was the salient feature of the sucrose/oil mixture. It is not clear if saliency of the corn oil results from its texture, taste, or odor. In previous experiments [14,15] the texture question was addressed by comparing the intake of corn oil and mineral oil in sham-feeding experiments. The purpose of the present experiment was to see if the rat could discriminate a sucrose/corn oil from a sucrose/mineral oil mixture. Assuming that the texture of corn and mineral oils is similar, if the rat makes this discrimination it would lend evidence to the idea that the salient feature of the corn oil in the mixture would be the result of taste or olfaction. In this experiment the corn oil/sucrose mixture was the CS in the flavor aversion design, and the postconditioning test was a preference test between a corn oil/sucrose mixture and a mineral oil/sucrose mixture.

6.1. Subjects

The subjects were 20 male rats, ranging in weight from 283 to 364 g.
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6.1. Subjects

The subjects were 20 male rats, ranging in weight from 283 to 364 g.
6.2. Procedure

After the standard water deprivation accommodation schedule, all of the rats received the corn oil/sucrose mixture for 15 min on conditioning day. Immediately after this period, half the rats received a LiCl and the other half got a saline injection. On the next 2 postconditioning test days all rats received a 1-h two-bottle preference test between 16% corn oil mixed with 0.25 M sucrose and 16% mineral oil mixed with 0.25 M sucrose.

6.3. Results

The mean consumption for the average consumption of the 2 days of testing can be seen in Fig. 5. The saline injected rats consumed an average of 7 mL of corn oil and 9 mL of mineral oil in the 2-h tests. This difference was not statistically significant (p > 0.09). The LiCl injected rats consumed an average of 2.5 mL of corn oil and 7 mL of mineral oil (p < 0.001). With the conditioned rats the intake of corn oil was significantly less than that of mineral oil within the first hour of testing. From the first to the second day of testing with the conditioned rats, the intake of corn oil remained quite low whereas mineral oil intake significantly increased from about 4.5 to 9.5 mL, indicating that the discrimination of corn oil from mineral oil was more robust with 2 days of testing.

6.4. Discussion

As was seen by Greenberg and Smith [15], Ackoff and Sclafani [1] and Ramircz [21], the control rats in this experiment seem to be indifferent to the type of oil, drinking similar quantities of the mineral and corn oils. When conditioned to the sucrose/corn oil mixture, they were able to make the discrimination between the two oils. This discrimination was made early in the 60-min test, so it is unlikely that postigestional factors played a major role. The outcome of this experiment leads to the conclusion that more than texture is involved in the saliency of the corn oil in the sugar solution. In the next experiment, methods are used to see just how rapidly the rat makes the discrimination of these two oils. If the discrimination is made within a few seconds in the first postconditioning test, the evidence will be strengthened that the discrimination does not depend on postigestional factors.

7. Experiment 5

In addition to the textural and possible taste and odor cues that the rat gets in the oral cavity when ingesting fats, it is well known that there is a significant postigestional contribution to fat intake [10–12,14,17,22,23,29]. In these present experiments, the preference tests were 60 to 90 min in duration, which allows ample time for postigestional factors to influence the intake of the sweet/fat mixtures. In addition, as has been pointed out earlier, the phase separation of the corn oil/sucrose mixtures results in proportionally lesser concentrations of corn oil over the 60- to 90-min periods. The purpose of this experiment was to determine if the salient feature of the corn oil/sucrose mixture would be the oil if very short-term ingestive tests were used. It is assumed that postigestional factors would play much less a role, if any.

7.1. Subjects

The subjects were 10 female rats weighing between 211 and 280 g.

7.2. Taste testing apparatus

The testing chamber was a “Davis Rig,” which is described in detail elsewhere [8,26]. A tray, holding eight bottles equipped with stainless steel drinking tubes, could be moved so that any one of the tubes was in alignment with a drinking port in front of the rat’s cage. The positioning of the particular tube was computer controlled by a reversible motor and a helipot under the tray. A motor-driven shutter occluded the drinking port except during the specified drinking periods.

7.3. Procedure

After the rat was placed in the testing cage and allowed to accommodate for a minute, the shutter was opened and the rat had access to the first drinking tube. The first lick on the tube (recorded by a contact circuit) initiated a 30-s drinking period, after which the shutter was closed and the second bottle was moved into position. Thirty seconds later the shutter opened again and the first lick initiated the second drinking period. This procedure was repeated until the rat had completed a 30-s drinking period on each of the eight tubes. Prior to conditioning the flavor aversion, the rats were given 10 daily training sessions in the apparatus with deionized water in each of the tubes. A thirsty rat can make 180–200 licks in each 30-s period, obtaining as much as 8 mL of fluid in the brief daily session.
On conditioning day, all eight tubes were filled with the corn oil/sucrose CS described in Experiment 1, and the rats were allowed to drink as described above. Following this session, half of the rats were injected with LiCl to condition the aversion and the other half served as controls, receiving an isotonic NaCl injection. For the next 2 days, the tubes were presented in sequence 1 through 8. Tube 1 contained water, tube 2 contained the corn oil/sucrose mixture, tube 3 contained 0.25 M sucrose, and tube 4 contained 16% corn oil. Tubes 5–8 were filled with the same solutions as tubes 1–4, respectively.

7.4. Results

A score was calculated for each rat based on the average number of licks that she made for water, for the mixture, for sucrose and for corn oil in presentations 1 and 5, 2 and 6, 3 and 7, and 4 and 8, respectively. The mean numbers of licks for the LiCl- and saline-injected rats are presented in Fig. 6. Here, it can be seen that the LiCl- and saline-injected rats make the same number of licks on the water tube in that 30-s period, t(8) = .08, p > 0.05. The LiCl-injected rats show the expected aversion to the corn oil/sucrose mixture, making only an average of 8 licks compared to the 148 licks for the saline injected group, t(8) = 6.64, p < 0.001. There is no significant aversion to the 0.25 M sucrose, t(8) = 0.91, p > 0.05. There is a significant aversion to the corn oil emulsion, t(8) = 2.11, p < 0.03.

7.5. Discussion

The results of this experiment are similar to the results from Experiment 1. The rats develop an aversion to the corn oil/sucrose mixture following a pairing of this mixture with a LiCl injection. This aversion generalizes to the corn oil component of the mixture, but not to the sucrose component. Because the ingestion in this experiment was limited to 30-s drinking periods compared to the 90-min tests in Experiment 1, one can conclude that it is very unlikely that post-ingestional consequences played any role in the development of this aversion. Furthermore, these tests were so short that it is very unlikely that the phase separation of the oil and water played any role in the magnitude of these aversions.

8. Experiment 6

The fatty acids that can be cleaved from corn oil are linoleic (about 60%), oleic (about 25%), palmitic (about 12%), and stearic (about 2%). Linoleic is an essential fatty acid that must be obtained from food. Gilbertson and colleagues [10–13] hypothesize that the lingual lipase from Von Ebner's gland cleaves linoleic and other fatty acids from the corn oil, and that the rat gets a gustatory sensation from these acids. The taste cells from which they recorded were sensitive to linoleic acid in concentrations as low as 10 μM. If this is one way that the rat receives orosensory input from corn oil, it should be possible to see generalization of a taste aversion from corn oil to linoleic acid and from linoleic acid to corn oil. These generalizations are shown in this experiment.

8.1. Subjects

Forty female rats, ranging in weight from 262–409 g, were divided into four groups of 10 each. They were housed and accommodated to the drinking schedule as has been previously described.

8.2. Procedure

On conditioning Day 20 the rats were given 16% corn oil as the CS and the other 20 were given a 22-μM concentration of linoleic acid. This concentration of linoleic acid was selected because it fell within the range of concentrations found by Gilbertson in his electrophysiological studies to stimulate a variety of taste cells. To prepare the linoleic acid solution, 28 μL of linoleic acid was mixed in 1.0 mL of ethanol using a vortex mixer for 1 min. This linoleic acid/ethanol solution was then mixed with the 1 L of dionized water. After a 15-min drinking period with these mixtures on conditioning day, the rats were subdivided into two groups, with one receiving the LiCl and the other receiving the saline injections. Forty-eight hours later they were given the preference tests. The rats that received corn oil as the CS were tested in a 1-h preference test with linoleic acid and water. One milliliter of ethanol was added to the water to ensure that the rats were discriminating the taste on the basis of the fatty acid and not the alcohol vehicle. The rats that received the linoleic acid as the CS were given their 1-h preference test with corn oil and water.

8.3. Results

The group mean intakes can be seen in Fig. 7. In the upper panel it can be seen that the linoleic acid/LiCl conditioned rats showed a significant aversion to the corn oil,
\( \tau(18) = 11.58, p < 0.001 \). Illustrated in the lower panel is the aversion to the linoleic acid in rats that had been conditioned with the corn oil/LiCl pairing, \( \tau(18) = 4.52, p < 0.001 \).

8.4. Discussion

With the taste aversion conditioning design it is possible to show that conditioning an aversion to corn oil generalizes to linoleic acid and vice versa. The assumption that there is a common orosensory factor for corn oil and linoleic acid that leads to these generalizations must be taken with some caution. Flavor aversion conditioning possibly can result in enhanced neophobia that would result in rejection of any solution that is novel to the rat. Therefore, it is possible that there would be generalizations to other taste compounds, but these have yet to be tested. In preliminary experiments we have demonstrated the generalization of the taste aversion from linoleic to oleic and palmitic acids, but not to arachidonic acid.

9. Experiment 7

From Experiments 1 and 2 we concluded that the most salient feature of a corn oil/sucrose mixture was the corn oil. If this saliency were based solely on gustatory sensation, it is quite possible that the salient feature of a sucrose/linoleic acid mixture would be linoleic acid. In the next experiment a sucrose/linoleic acid mixture was used as the CS, and the preference tests were conducted with (a) sucrose/linoleic acid versus water, (b) sucrose versus water, and (c) linoleic acid versus water in a similar pattern to that used in Experiment 1 with corn oil mixtures. Finally, a test was run between sucrose and linoleic acid.

9.1. Subjects

The subjects were 20 female rats, ranging in weight from 237–253 g. They were housed and accommodated to the drinking schedule as in the previous experiments.

9.2. Procedure

On conditioning day all rats received 15-min access to a mixture of 0.25 M sucrose and 22 \( \mu \)M linoleic acid. Immediately following this drinking period, 10 of the rats received the LiCl and 10 received the saline injection. Twenty-four hours later on the first testing day the rats received a 1-h preference test between the sucrose/linoleic acid mixture and water. On the second testing day the preference test was between sucrose and water, and on the third testing day the choice was between linoleic acid and water. In all of these preference tests ethanol was added to the water to ensure that the rats were responding to the fatty acid and not the ethanol vehicle. On the final testing day the rats had a preference test between sucrose and linoleic acid.

9.3. Results

The results of the preference tests for the first 3 postconditioning days are illustrated in Fig. 8. It can be seen in the upper panel that there was a significant aversion conditioned to the linoleic/sucrose mixture, \( \tau(18) = 8.06, p < 0.001 \). The aversion to sucrose, which is shown in the middle panel, is also significant, \( \tau(18) = 24.35, p < 0.001 \). The aversion to the linoleic acid is not as large as that to the sucrose, but it is significantly different from the saline injected group, \( \tau(18) = 3.02, p < 0.007 \). The results of the preference test between sucrose and linoleic acid are seen in Fig. 9. The LiCl-injected rats exhibited a strong aversion to the sucrose, \( \tau(18) = 7.69, p < 0.001 \), while the saline-injected rats had a strong preference for sucrose over linoleic acid, \( \tau(18) = 7.92, p < 0.001 \).

9.4. Discussion

Unlike the results from Experiment 1, the stronger conditioned taste aversion in this experiment was to the sucrose. The salient feature of the sucrose/linoleic acid mixture is not the linoleic acid. The fact that the rats developed an aversion to the linoleic acid, after the sucrose/linoleic acid was used as the CS, indicates that the rats could detect the presence of the acid in the mixture.
conditioned compared to sucrose, because the magnitude of the aversion to sucrose or corn oil alone is similar. There is evidence in the taste aversion literature that the magnitude of the CTA can be affected by the quality and the concentration of the taste stimulus [31]. Further evidence that the salient orosensory quality of the corn oil/sucrose mixture was the oil was demonstrated in Experiment 3, where conditioning with the corn oil as the CS generalized so profoundly to the corn oil/sucrose mixture. This aversion was very strong over a 5-day testing period. In contrast, when the sucrose was used as the CS, there was a more modest aversion to the corn oil/sucrose mixture, but this aversion was completely abated over the 5-day testing period (see Fig. 4). The salience of the corn oil in the mixture demonstrated in the short-term testing periods (Experiment 5) makes it unlikely that postigestional factors played a role in this discrimination. On the second day after using the corn oil/sucrose mixture as a CS, in 30-s single-bottle tests the conditioned rats drank normal amounts of water and sucrose, but markedly reduced their intake of the corn oil/sucrose mixture and corn oil when compared to their saline-injected controls.

In Experiment 4 it was shown that with CTA the rat could discriminate between sucrose mixed with corn oil and sucrose mixed with mineral oil. From visual observations of the rats, the drinking occurred very early in the 60-min preference test, so it is unlikely that postigestional factors played any role in this discrimination. This finding would agree with the two-bottle sham-feeding experiment of Greenberg and Smith [15], where they found a strong preference of corn oil over mineral oil in a test where postigestional factors played no role. However, the saline-injected rats in the present experiment seemed not to be able to discriminate between the two oils when mixed with the strong sucrose solution. It is possible that with further testing a preference for corn oil/sucrose over mineral oil/sucrose would have developed. The discrimination that the rats
made between the two oil solutions could have been on the basis of taste or possibly olfaction or viscosity [21].

Some evidence that taste plays a role in the orosensory perception of corn oil was given in Experiment 6, where the rats generalized from corn oil to linoleic acid and vice-versa. If the lingual lipase in the rat’s mouth initiates the breakdown of corn oil into its fatty acid components, linoleic acid would be a likely candidate for a gustatory input [9–13]. Again, without a comprehensive study of a wide variety of concentrations, a firm conclusion regarding the role of gustation in the perception of fat by the rat is premature. At the present time, using CTA designs, we are studying a “cocktail” of the principal fatty acids derived from corn oil (linoleic, oleic, and palmitic) to see if this cocktail is specific to corn oil compared to others oils.

If gustation plays a role in the perception of corn oil by the rat, it is certainly only part of the story. The results of the last experiment in this series show that linoleic acid does not emerge as the salient feature of a sucrose/linoleic acid mixture as corn oil did in the sucrose/corn oil mixture. The rats can discriminate the presence of the linoleic acid in the mixture, but it does not dominate the orosensory perception in the same manner as corn oil. The possible textural and olfactory features of corn oil make it quite different from linoleic acid as an orosensory stimulus. In preliminary work, we have repeated the last experiment with increased concentrations of linoleic acid and have obtained essentially the same data as reported here.

The relative roles of texture, olfaction, and gustation in the orosensory perception of fats to the rat need further investigation. At present, we are testing rats with olfactory bulb ablations in similar experiments as those reported here. The present experiments, combined with the electrophysiological work of Gilbertson and colleagues [9–13], suggest that there may be a role for gustation in the ingestion of fat by the laboratory rat.

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