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Food Deprivation Increases the Rat's Preference for a Fatty Flavor Over a Sweet Taste

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Abstract

Previous research indicates that food deprivation increases the rat's preference for high-fat over low-fat foods. Since these foods differ in their flavors and post-ingestive effects, both factors may be implicated. The present study investigated preferences in food deprived and non-deprived rats using non-nutritive mineral oil emulsion (MO) and saccharin solution (SAC), which have a fatty flavor and sweet taste, respectively. The deprived rats consumed more MO than SAC in one- and two-bottle tests, while the non-deprived rats ingested as much SAC as MO in one-bottle tests and preferred SAC in two-bottle tests. Several aspects of the data suggest that the deprivation-related shift in preference between MO and SAC was determined by changes in long-term energy balance. A follow-up conditioning experiment discarded the possibility that the observed preference shift was related to differential reinforcing effects of the two substances. In conclusion, long-term food restriction increases the preference for an oily flavor over a sweet taste via a mechanism that does not involve nutritive feedback. It remains to be determined to what extent this alteration in flavor preference influences food selection when post-ingestive nutritive feedback can influence food choice. **Chem. Senses 21: 169–179, 1996.**

Introduction

Food deprivation, in addition to increasing subsequent food consumption also alters food preferences. In particular, several studies report that deprivation increases the selection of high-fat foods in rats allowed to self-select their diet from different food sources (Schutz and Pilgrim, 1954; Andik *et al.*, 1971; Piquard *et al.*, 1978; Reed *et al.*, 1988; Tempel *et al.*, 1989; Bligh *et al.*, 1990; Gerado-Gettens *et al.*, 1991; Bernardini *et al.*, 1993). To account for this effect various hypotheses have been put forward. It has been proposed, for example, that hungry rats select fat because of its increased caloric density relative to carbohydrate and protein (Schutz and Pilgrim, 1954; Andik *et al.*, 1971; but see Bernardini *et al.*, 1993), or because their metabolic state favors fat utilization over carbohydrate utilization (Piquard *et al.*, 1978, 1979; Bligh *et al.*, 1990). However, little experimental work has been devoted to the mechanisms underlying deprivation-induced increase in fat appetite, which is surprising given its potential importance to human fat intake and dieting behavior. Recent work in our laboratory indicates that deprivation may enhance fat appetite at least in part by increasing the hedonic response to the flavor properties of fat (Sclafani and Ackroff, 1993). [Note that, as used here, the term 'flavor' refers to olfactory, gustatory and tactile cues taken together (McBurney, 1986).] When given short-term choice-tests with 2% sucrose and 0.9% corn oil, non-deprived rats consumed 27% of their total

intake as corn oil, whereas food deprived rats took 81% of their intake as corn oil. A similar, but less dramatic preference shift was observed in rats tested with 2% polycose and 0.9% corn oil. Given that the nutrient sources were isocaloric and rather dilute (0.08 kcal/g), the deprivation-induced preference changes appeared to be mediated by the orosensory rather than the post-ingestive properties of the nutrients. However, a role for post-ingestive factors cannot be completely ruled out particularly since our laboratory has reported that flavor preferences can be conditioned by intragastric infusions of dilute nutrients (e.g. 2% polycose) (Ackroff and Sclafani, 1994). The present study further investigated the role of flavor cues in deprivation effects on fat and sugar preferences. This was accomplished by using mineral oil and saccharin which have been used in prior animal studies as non-nutritive substitutes for sucrose and corn oil, respectively (Collier and Novell, 1967; Carlisle and Stellar, 1969; Ackroff *et al.*, 1990; Mindell *et al.*, 1990).

Experiment 1

Methods

Subjects

The subjects were 16 female adult Sprague–Dawley derived rats born in our laboratory from Charles River Laboratories (Wilmington, MA) CD stock. The animals were individually housed in standard wire-mesh cages in a vivarium maintained at 21°C under a 12:12 h light:dark cycle (lights on at 8:00 a.m.). Body weights ranged from 204 to 281 g at the beginning of the experiment. Purina Chow (No. 5001) and tap water were available *ad libitum* except where noted.

Stimuli and tests

Solutions of 0.05% or 0.2% (w/w) sodium saccharin (Sigma Chemicals, St Louis, MO) were made using tap water. Mineral oil (Squibb, Princeton, NJ) (10% w/w) emulsions were prepared using tap water and 0.2% (w/w) Emplex (Patco, Kansas City, MO) as an emulsifier. The stimulus concentrations were chosen to approximate the hedonic value of 0.9% corn oil and 2% sucrose, which were used in our previous work (Sclafani and Ackroff, 1993). Mineral oil is less preferred than iso-concentrated corn oil (Mindell *et al.*, 1990), and in a pilot study we determined that rats equally preferred 10% mineral oil and 0.9% corn oil. Prior work indicates that a 0.2% saccharin concentration is isohedonic to 2% sucrose (Young and Madsen, 1963; Collier and Novell, 1967). The oil emulsion was prepared by adding the oil and emulsifier to hot water (160–170°F) and homogenizing the mixture at high speed with an Ultra-Turrax T-25 (Ika-Works Inc., Cincinnati, OH). The emulsion was then cooled in a cold water bath. The saccharin solution and oil emulsion were prepared fresh before each test and were served at room temperature. They were presented in 50-ml graduated tubes and intakes were recorded to the nearest 0.5 ml.

One-bottle acceptance tests and two-bottle preference tests (30 min/day) were conducted about 4 h into the light period, after the daily weighing of the rats. During the one-bottle testing, the stimuli were presented over consecutive test sessions using an ABAB order; for each stimulus, the bottles were placed on the left or right sides of the cages an equal number of times. During the two-bottle tests, the drinking tubes were presented 10–12 cm apart and the left-right position of the stimuli was alternated daily.

Procedure

Experiment 1A

The rats were divided into two groups (n = 8 each) matched for body weight. One group (AL) received chow and water *ad libitum* except during the tests and the hour following the tests. The rats in the other group (DEP) were food restricted and maintained at 85% of their *ad libitum* body weight; daily food rations were given 1 h after the tests. Both groups were first tested in one-bottle sessions with 0.05% saccharin and 10% mineral oil (five sessions/stimulus). This was followed by a two-bottle test (four sessions) with the same test stimuli. The rats were then given a one-bottle test (four sessions/stimulus) with the 0.2% saccharin solution and the 10% mineral oil emulsion followed by a two-bottle test (four sessions).

Experiment 1B

The rats from Experiment 1A were given an additional two-bottle test (four sessions) with 0.2% saccharin versus 10% mineral oil after being 'prefed'. That is, 1 h before the sessions, the DEP rats received their food ration and the AL rats were given fresh food; note that the food had been given after the tests in Experiment 1A. An hour later, the remaining food was taken from the cages and weighed, and the preference test started. After the test sessions, the DEP rats received the remainder of their daily ration, while the AL rats were given unlimited chow.

Experiment 1C

Following Experiment 1B, the groups had their feeding conditions reversed. The rats that were fed *ad libitum* were now food-restricted (group NEW DEP); their body weights were brought to 85% of the *ad libitum* value and maintained at this level by giving them restricted chow rations. The rats previously food deprived were now fed *ad libitum* (NEW AL group). Seven days after the reversal in feeding conditions, testing with 0.2% saccharin and 10% mineral oil resumed. The rats were given a two-bottle test (four sessions), followed by a one-bottle test (3 sessions/stimulus) and then an additional two-bottle test (four sessions).

Statistical analysis

Intakes were entered as repeated measures in analyses of variance (ANOVA), followed by simple main effects when appropriate. Deprivation level was a between- or within-factor depending on the analysis; test stimulus, test type and test day (or block) were within-factors.

Results

Experiment 1A

During the first one-bottle test (0.05% saccharin, 10% mineral oil), intakes increased over sessions in all rats, and then were stable during the last two sessions with each stimulus. During these last four sessions, stimuli intakes were identical in the AL rats, but the DEP rats drank significantly more mineral oil emulsion than saccharin solution [Figure 1; group×stimulus: F (1,14) = 65.6, $P <$ 0.001]. Intake of saccharin did not differ between groups, whereas mineral oil intake was greater in the DEP than in the AL group [F (1,25) = 90.9, $P < 0.001$]. During the following two-bottle test (Figure 1), the AL rats consumed similar amounts of the two stimuli, whereas the DEP group consumed substantially more mineral oil than saccharin; [group×stimulus: F (1,14) = 43.7, $P < 0.001$]. The outcome with a higher concentration of saccharin (0.2%) was similar (Figure 1). That is, one-bottle intake of mineral oil was much greater in the DEP than in the AL group [F (1,21) = 114.5, $P < 0.001$], while deprivation did not increase saccharin intake significantly [deprivation×stimulus: F (1,14) = 96.3, $P < 0.001$]. In the two-bottle test, the DEP rats consumed more mineral oil [F (1,14)=19.4, $P < 0.001$], while the AL rats tended to consume more saccharin ($P < 0.11$) [deprivation×stimulus: F (1,14) = 18.7, $P <$ 0.001]. The percentage intake of mineral oil was 70 and 30%, respectively, for the DEP and AL groups.

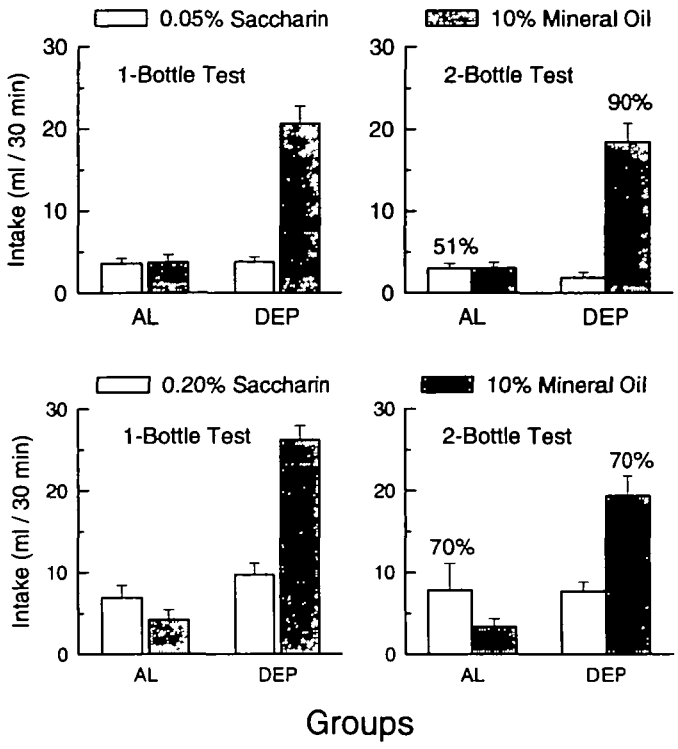

Figure 1 Experiment 1. Mean (+SE) intakes of mineral oil emulsion (10%) and saccharin solution (top: 0 05%, bottom: 0 20%) in one-bottle (left, average of last 2 sessions/stimulus) and two-bottle 30-min tests (right, average of 4 sessions). The AL group was fed *ad libitum* and DEP group was food deprived. The numbers above bars represent the percentage of total intake consumed as saccharin or mineral oil

Experiment 1B

When the rats were allowed to feed for an hour before the test, the DEP rats consumed substantially more chow than did the AL rats [9.7 versus 1.3 grams, t (14) = 12.5, $P < 0.001$]. Prefeeding the rats before the tests did not change the preference pattern from that seen in the previous experimental phase. The DEP rats continued to strongly prefer (76%) mineral oil to saccharin [14.2 versus 5.1 ml/30 min; F (1,14) = 19.3, $P < 0.001$] and the AL rats strongly preferred (80%) saccharin [9.0 versus 2.0 ml/30 min; F (1,14) = 11.5, $P < 0.01$] [group×stimulus: F (1,14) = 30.3, $P < 0.001$].

Experiment 1C

After the feeding conditions were reversed, the two-bottle test revealed a change in the preference pattern (see Figure 2). The NEW AL group consumed more saccharin than mineral oil, whereas the NEW DEP group consumed equal

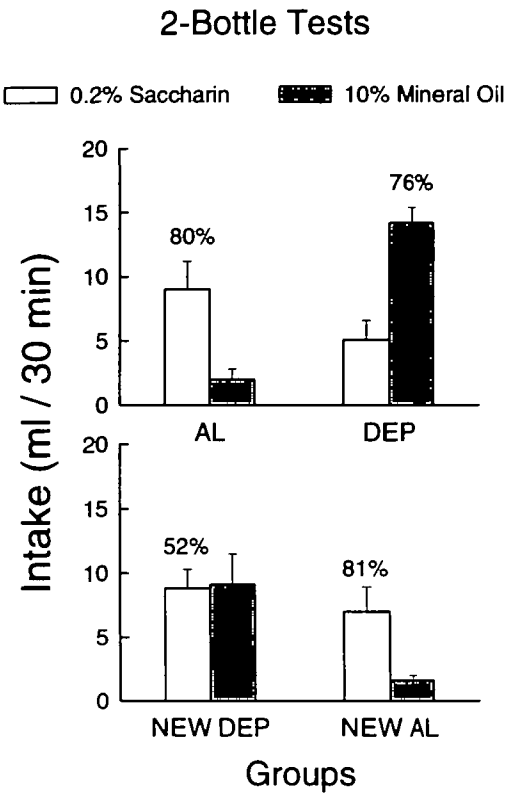

Figure 2 Experiment 1 Mean (+SE) two-bottle intakes of mineral oil emulsion and saccharin solution before (top, 'prefed' test, four sessions) and after (bottom, four sessions) reversal of the dietary conditions. One group (left) was first fed *ad libitum* (AL) and then deprived (NEW DEP). The other group (right) was first deprived (DEP) and then fed *ad libitum* (NEW AL) The numbers above bars represent the percentage of total intake consumed as saccharin or mineral oil.

amounts of the two stimuli although this difference was marginally significant [group×stimulus, F (1,14) = 4.2, $P < 0.06$]. The effects of the dietary switch on two-bottle intakes was also analysed using within-group comparisons. Looking at the last session of 'prefeeding' tests before the switch and the first session after the switch revealed clear changes in preferences. The rats that were initially food-restricted and then fed *ad libitum* changed from preferring saccharin (73%, $P < 0.001$) to tending to prefer mineral oil [73%, $P < 0.11$; deprivation×stimulus: F (1,6) = 32.3, $P < 0.002$; data from one rat that did not drink on the first *ad libitum* session were not used]. Conversely, the rats that were first fed *ad libitum* and subsequently deprived changed from preferring saccharin (84%, $P < 0.05$) to being indifferent [deprivation×stimulus: F (1,7) = 5.84, $P < 0.05$].

In the following one-bottle tests, the NEW DEP rats consumed more mineral oil emulsion than saccharin solution [18.0 versus 12.4 ml/30 min; F (1,14) = 7.0, $P < 0.05$] while the NEW AL rats consumed slightly less oil emulsion than saccharin solution [6.3 versus 7.4 ml/30 min; group×stimulus: F (1,14) = 5.0, $P < 0.05$]. When the rats were tested again in a two-bottle situation, the results were similar to the first series of tests: the NEW DEP rats drank slightly more oil emulsion than saccharin solution (14.4 versus 11.5 ml/30 min), while the NEW AL rats preferred 79% saccharin over mineral oil [12.4 versus 2.8 ml/30 min, F (1,14) = 10.5, $P < 0.01$; group×stimulus: F (1,14) = 9.0, $P < 0.01$].

Discussion

The findings show that food deprivation increases the acceptance of and preference for a mineral oil emulsion over a saccharin solution. Similar results were obtained with two saccharin concentrations (0.05 and 0.20%), although they were more clear-cut with the higher saccharin concentration. The present results are consistent with the prior findings that food deprivation increases the preference for 0.9% corn oil suspension over a 2% sucrose suspension (Sclafani and Ackroff, 1993). We have also obtained comparable results with mineral oil and saccharin presented as suspensions using xanthan gum (F. Lucas and A. Sclafani, unpublished observations). Since the present experiment used non-nutritive substances, the rats' choices were not influenced by nutritive post-ingestive cues from the test stimuli.

The increased mineral oil preference in the deprived rats does not appear to be related to immediate repletion/depletion signals (i.e. presence/absence of food in the gut), but rather to long-term energy state. When given access to food for an hour just prior to the tests, the DEP rats ate a fairly large meal (about 10 g, corresponding to 70% of their daily ration), but their mineral oil preference was not altered. However, the same rats (NEW AL) allowed to refeed freely on chow for 1 week before being tested displayed a dramatically reduced mineral oil preference (from 76 to 21%). Conversely, the rats (NEW DEP) switched from *ad libitum* feeding to a food deprivation schedule increased their preference for mineral oil (from 20 to 52%), although they did not consume more mineral oil than saccharin as did the DEP group in Experiment 1A.

Experiment 2

Experiment 1 showed that, relative to saccharin, deprived rats preferred mineral oil more than replete rats. This difference was observed after the rats had received one-bottle tests with these stimuli. It is possible that during this

initial exposure the rats experienced differential positive or negative consequences of consuming saccharin and mineral oil depending upon the deprivation state. In particular, it has been proposed that consuming saccharin in the deprived state has aversive effects due to 'unsatisfied' cephalic reflexes (Tordoff and Friedman, 1989b). It could be, therefore, that both *ad libitum* and deprived rats initially prefer saccharin to mineral oil, but that one-bottle experience leads deprived rats to acquire a mild aversion (or reduced preference) for saccharin. Note that this interpretation is not supported by the results obtained in Experiment 1C. That is, in the first preference test after the reversal in feeding condition, the formally deprived rats (NEW AL) who had previously avoided saccharin in preference to mineral oil, now strongly preferred saccharin (80%). Nevertheless, the present experiment investigated the role of learning more directly by measuring the preference for saccharin and mineral oil in rats naive to these stimuli, i.e. without previous one-bottle training. During the first choice sessions the effect of learning, if any, should be minimal. In order to insure the rats would consume adequate amounts during their first saccharin versus mineral oil test, they were first trained to drink in short-term sessions using dilute polycose solutions. Polycose was used because it has a palatable taste to rats that differs from the sweet taste of saccharin (Nissenbaum and Sclafani, 1987) and presumably from the oily flavor of mineral oil.

Experiment 2 also further examined the change in saccharin versus mineral oil preference produced by reversal in feeding conditions. Experiment 1B showed that switching the feeding conditions reversed the rats' preference. However, the animals were not tested until the seventh day after the reversal in deprivation state and thus it is not known how soon after being deprived (or refed) animals change their flavor preferences. This question is of interest in light of the results obtained with the 1 h prefeeding manipulation of Experiment 1B. In Experiment 2B, therefore, the rats were given two-bottle tests starting 1 day after the reversal in feeding conditions.

Methods

Experiment 2A

Sixteen new rats were used with body weight ranging from 203–281 g at the beginning of the experiment. The subjects' description and the experimental procedure were identical to those described for Experiment 1, except for the following differences. After being placed on their respective feeding regimes, the DEP and AL groups were trained to drink during 30-min, two-bottle tests with polycose and water; they received 4% polycose (Ross Laboratories, Columbus, OH) versus water (seven sessions), then 4 versus 2% polycose (2 sessions), then 2% polycose versus water (2 sessions). In the experiment proper, the rats were first given a two-bottle test with 0.2% saccharin solution versus 10% mineral oil emulsion (two sessions), followed by a one-bottle test (five sessions/stimulus) and then a second two-bottle test (10 sessions).

Experiment 2B

At the end of Experiment 2a, the feeding conditions of the two groups were reversed; the former *ad libitum* group was now food deprived (NEW DEP group), and the former deprived group was now fed *ad libitum* (NEW AL group). Two-bottle testing with saccharin versus mineral oil started on the day following the switch in feeding condition and continued for eight daily sessions.

Results

Experiment 2A

As shown in Figure 3, both DEP and AL groups strongly (78–80%) preferred the mineral oil emulsion over the saccharin solution in the initial two-bottle test [F (1,14) = 23.6, $P < 0.001$]; there was no between-group difference in preference. In the following one-bottle test, intakes of saccharin progressively increased over sessions in both groups, while mineral oil intake remained unchanged (stimulus×days: F (4,56)=7.24, $P < 0.001$]. Intakes stabilized by the last two sessions with each stimulus and the DEP group consumed more mineral oil [F (1,21) = 28.3, $P < 0.001$] and saccharin [F (1,21) = 6.3, $P < 0.05$] than did the AL group. The DEP rats consumed more mineral oil than saccharin [20.9 versus 12.0 ml/30 min, F (1,14) = 28.2, $P < 0.001$], while the AL rats' mineral oil intake was not reliably greater than their saccharin intake [7.5 versus 5.7 ml/30 min; group×stimulus: F (1,14) = 8.9, $P < 0.01$]. The two-bottle data (averaged in 2-day blocks) following the one-bottle test are displayed in Figure 3. In the first 2-day block the DEP rats showed a strong (86%) mineral oil preference ($P < 0.001$), while the AL rats displayed no preference [group×stimulus: F (1,14) = 47.8, $P < 0.001$]. As compared to the choice tests before the one-bottle test, the mineral oil preference had decreased in the AL rats and increased in the DEP rats [group×stimulus×test: F (1,14) = 36.7, $P < 0.001$]. Mineral oil preference decreased across

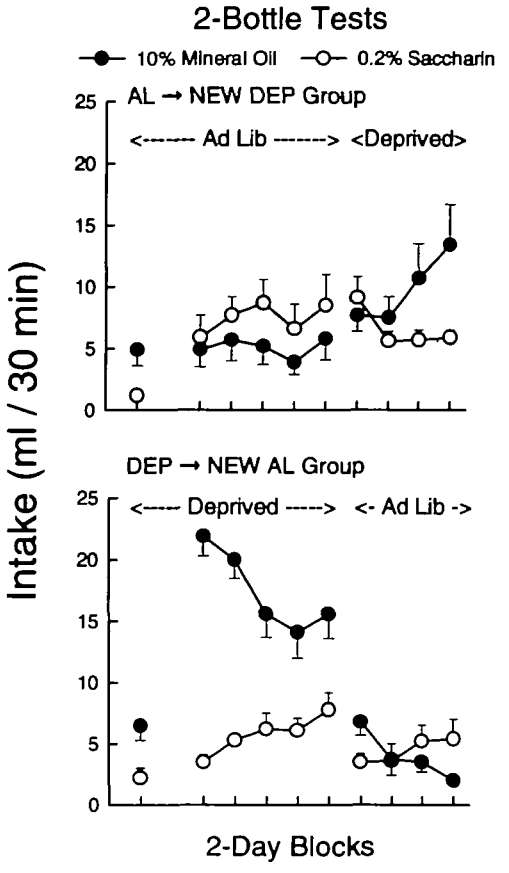

Figure 3 Experiment 2 Mean (+SE) two-bottle intakes of mineral oil emulsion (10%) and saccharin solution (0.2%) during 30-min sessions averaged over 2-day blocks The rats were given one-bottle test sessions between blocks 1 and 2 (data not shown). One group (top) was first fed *ad libitum* (AL) and then deprived (NEW DEP). The other group (bottom) were first deprived (DEP) and then fed *ad libitum* (NEW AL).

2-day blocks in the DEP group, but did not change significantly in the AL group [group×stimulus×block: F (4,56) = 3.18, $P < 0.02$]. During the last block, however, the DEP group consumed more ($P < 0.05$) mineral oil than saccharin, while the AL group tended to consume more saccharin than mineral oil [group×stimulus: F (1,14) = 5.55, $P < 0.05$].

Experiment 2B

When the feeding conditions were reversed, preferences did not change immediately (Figure 3). In the first 2-day block, the mineral oil preference was comparable to the preswitch value in both groups (NEW AL: 66 versus 66%; NEW DEP: 46 versus 41%) and, consequently, the mineral oil preference was greater in the NEW AL rats than the NEW DEP rats [group×stimulus: F (1,14) = 7.4, $P < 0.02$]. However, preferences changed over successive 2-day blocks in ways that differed between groups [group×stimulus×blocks: F (3,42) = 18.8, $P < 0.001$]. That is, the NEW AL rats changed from preferring mineral oil [first block, F (1,26) = 9.3, $P < 0.01$] to preferring saccharin [last block, F (1,26) = 10.5, $P < 0.01$; stimulus×block: F (3,21) = 9.1, $P < 0.001$]. Conversely, the NEW DEP rats increased their oil preference from 46 to 61% from the first to last test blocks [stimulus×test block: F (3,21) = 9.9, $P < 0.001$]. The mineral oil preference was significantly greater in the NEW DEP than in the NEW AL in the third ($P < 0.05$) and fourth ($P < 0.02$) test blocks. Note that the body weights of the two groups had stabilized by the last block (data not shown).

Discussion

Experiment 2A examined preferences for mineral oil and saccharin in rats naive to these stimuli. In the initial two-bottle test, the two groups showed similar strong preferences for mineral oil emulsion. Therefore, deprivation state did not affect preferences in this test. However, a deprivation effect was obtained after one-bottle exposure. The DEP rats continued to prefer mineral oil while the AL rats drank slightly more saccharin than oil. Why one-bottle exposure produced these deprivation-related preference changes is not clear. The most notable change occurred in the non-deprived rats that lost their preference for mineral oil and tended to prefer saccharin. These results clearly do not support the idea suggested above that *ad libitum*, and deprived rats initially prefer saccharin and with experience deprived rats come to prefer mineral oil.

Conceivably, the rats' initial preferences (particularly in the non-deprived group) may have been related to their prior experience with polycose solutions in the preliminary phase of the experiment. A follow-up experiment tested this possibility: naive rats (n = 16) were fed *ad libitum* or restricted, and were trained to drink a mixture of saccharin (0.1%) and mineral oil (5%), for 30-min/day before being tested for their preference for mineral oil (10%) and saccharin (0.2%). The rationale for using the saccharin/mineral oil mixture was that if the rats experienced 'positive' or 'negative' effects during the initial training period, they would be related to both the flavors of saccharin and mineral oil. In the subsequent choice test both deprived and *ad libitum* groups strongly preferred the mineral oil emulsion (85 and 81% oil preference, respectively). Therefore, the initial mineral oil preference observed in Experiment 2A was not due to pretraining with polycose *per se*.

Experiment 2B showed that when feeding conditions were switched, a preference reversal did not occur immediately, but developed over the course of days. This contrasts with the results of Experiment 1C; in that experiment the rats

showed a preference reversal in the very first choice test, which occurred 1 week after the switch in feeding conditions. These data indicate that the gradually developing preference reversal in Experiment 2B was not due to repeated testing, but was more likely related to the rats' changing body energy stores. Consistent with this interpretation, the preference reversal was greatest at about the time the rats had reached stable body weights.

Experiment 3

Experiments 1 and 2 revealed that following one-bottle exposure to saccharin and mineral oil, *ad libitum* and deprived rats display opposite preferences for these stimuli. Since the groups did not differ in Experiment 2 prior to one-bottle training, it would appear that some aspect of the one-bottle experience alters the relative preference for saccharin and mineral oil. The rewarding/aversive properties of drinking saccharin in various deprivation states have been previously examined in a series of conditioning studies by Capaldi (Capaldi and Myers, 1982; Capaldi *et al.*, 1983; Campbell *et al.*, 1987). In brief, rats were given flavored saccharin solutions to drink in daily short-term sessions; one flavor was presented when rats were sated and another flavor when they were hungry. At the end of this one-bottle training, the rats avoided the flavor paired with the hungry state (and/or preferred the flavor paired with the sated state) in two-bottle tests. A similar phenomenon may have occurred in Experiment 1: as a result of one-bottle experience, the food-restricted rats may have formed a conditioned reduction in their liking for saccharin and/or the replete rats may have acquired an increased liking for saccharin. Accordingly, the preference for mineral oil over saccharin would have come to be greater in the deprived rats, compared to the non-deprived animals. However, this explanation would hold only if drinking mineral oil in deplete and replete states produced no (or less) conditioned change in liking. We tested this hypothesis using a conditioning paradigm similar to that of Capaldi. One group of rats was trained to drink flavored saccharin when hungry and a differently flavored saccharin when replete. A second group was trained similarly except that they received flavored mineral oil emulsions.

Methods

Sixteen new rats (225–256 g) of the same description as in Experiment 1 were used. They were placed on an alternating feeding/fasting schedule. That is, *ad libitum* chow was available every other day at 1600 h until the next day at 1030 h. After 12 days of adaptation to this schedule, the experiment proper started. Daily 30-min drinking sessions were run starting at 11:00 h so that the rats were alternately sated (0.5 h without food) and hungry (24.5 h without food) at the beginning of the session. The rats were divided into two groups matched for their body weight and food intake as measured during the feeding adaptation period. The SAC group (n = 8) received flavored saccharin (0.2%) solutions, while the MO group (n = 8) received flavored mineral oil (10%) emulsions. The flavors were grape and cherry Kool-Aid (0.05%, General Foods, White Plains, NY), which have been routinely used in conditioning experiments in our laboratory. During 20 one-bottle training sessions, the rats received 5 ml of the appropriate flavored fluid; rats were hungry for 10 sessions and sated for 10 sessions. Half the rats received grape when sated and cherry when hungry; the pairings were reversed for the remaining rats. In the next eight daily sessions (four when sated and four when hungry) the subjects were given a two-bottle choice between the two flavored fluids. The left-right position of the flavored fluids was alternated every 2 days during the training and the test phases.

Results

Before the start of the feeding schedule, the rats' mean food intake and body weight were 20 g/day and 244 g, respectively. On the alternating feeding schedule, the rats stabilized their food intake at about 28 g/2 days. When replete the body weights of the two groups (237, 240 g) were close to their *ad libitum* value; when deprived, their mean body weight dropped to 200–206 g (about 84% of the *ad libitum* body weight).

The two-bottle preference data, averaged over four trials, are shown in Figure 4. Both the SAC and MO groups drank more of the flavor previously experienced when sated than of the flavor experienced when hungry. The preferences ranged from 58 to 66% and were displayed when the rats were tested replete [F (1,14) = 6.5, $P < 0.05$] and deprived [F (1,14) = 15.4, $P<0.01$]; the differential intakes of the two flavors were greater in the deprived tests than in the replete tests [flavor×test deprivation: F (1,14) = 7.5, $P < 0.05$]. The MO group drank more fluid than the SAC group [F (1,14) = 22.6, $P < 0.001$], but the two groups did not differ in their preference for the flavor consumed when sated.

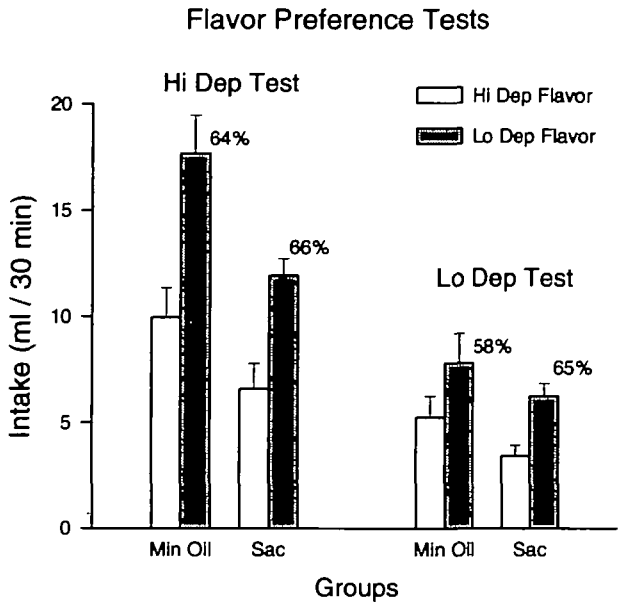

Figure 4 Experiment 3. Mean (+SE) intakes of flavored 10% mineral oil emulsion (Min Oil Group) and flavored saccharin solution (Sac Group) during 30-min two-bottle tests (four sessions). The rats had previously experienced one flavor when food deprived (Hi Dep Flavor) and another flavor (Lo Dep Flavor) when sated; the flavors were grape and cherry Kool-Aid. Preferences were tested when deprived (Hi Dep Test) or sated (Lo Dep Test). The numbers above bars represent the percentage of total intake consumed as the Lo Dep flavor

Discussion

These results replicate the previous finding that a flavor associated with drinking saccharin when non-deprived comes to be preferred to a flavor associated with drinking saccharin when deprived (Capaldi and Myers, 1982; Capaldi *et al.*, 1983; Campbell *et al.*, 1987). The strength of the conditioned preferences (58–66%) is close to the 60–65% preference that can be calculated from several of Capaldi's studies using comparable training and testing procedures (Capaldi and Myers, 1982; Capaldi *et al.*, 1983; Campbell *et al.*, 1987). The present experiment further demonstrates that rats also learn to prefer a flavor associated with drinking mineral oil when non-deprived over a flavor associated with drinking mineral oil when deprived, and the acquired preference was comparable in magnitude to that observed with saccharin-paired flavors. In view of these latter findings, the different preference profiles of non-deprived and deprived rats in Experiments 1 and 2 cannot be attributed to differences in the reinforcing properties of the stimuli under deprived and non-deprived states. Rats appeared to prefer both mineral oil and saccharin more when sated than when deprived. Nevertheless, when given the choice between the two substances, they preferred saccharin more when non-deprived and mineral oil more when deprived.

Note that Capaldi (1993) has reported that while rats learned to prefer flavors consumed with sweet solutions and food when sated, similar effects were not obtained with non-sweet foods. The mineral oil data of the present experiment demonstrate that with some non-sweet substances, deprivation-dependent learned flavor preferences can be as robust as with sweet substances. Related findings have been reported by Ramirez (1993a). In his experiments, rats were given preference tests with dilute corn oil (0.5–0.9%) suspensions versus vehicle while food sated (days 1, 2 and 4) and deprived (day 3). He reported that oil preference was somewhat increased on deprivation days, but was clearly decreased on the post-deprivation day. Similar tests using dilute carbohydrate (1% sucrose, 2% polycose, 1–2% starch) suspensions failed to reveal a post-deprivation reduction in preference. The possibility that the decrease in oil preference was due to a conditioned aversion was tested by adding a cue flavor to the oil suspension (but not the vehicle) during the non-deprived and deprived tests. Subsequently, the rats avoided the cue flavor even when the oil was no longer present. Thus, with Ramirez's test protocol, consuming oil when food deprived appears to have an aversive consequence that is not evident with dilute carbohydrate solutions. The nature of this aversive consequence is unknown. It has been argued that the sweet taste of saccharin or dilute sugar solutions elicits cephalic-phase digestive responses that are unsatisfied by the solution and this conditions a reduction in the reward value of sweet taste (Capaldi and Myers, 1982; Tordoff and Friedman, 1989a). Little is known about cephalic responses elicited by an oily flavor, and a similar mechanism could conceivably occur with ingestion of mineral oil.

General discussion

In Experiment 1 food-deprived rats showed an increased acceptance of and preference for a mineral oil emulsion relative to a saccharin solution while food sated rats displayed an opposite preference profile. These results agree with a previous report from our laboratory (Sclafani and Ackroff, 1993) using dilute corn oil and sucrose suspensions. Since the present study used non-nutritive substances, the deprivation state effect appears to be related to the orosensory properties rather than the post-ingestive effects of the test stimuli.

While the sweet taste of sugar and saccharin has been extensively studied, relatively little is known about the

orosensory cues that mediate fat sensation and preference. With respect to fats in the liquid form, such as oil emulsions/suspensions or fluid dairy products, texture (mouth feel) is usually thought to play a major role in identification and preference. Among the textural cues involved, viscosity has often been emphasized; however, viscosity appears to be only loosely tied to other important textural characteristics of fats (e.g. 'smoothness', 'creaminess', 'lubricating effect') (Mela, 1988; Mela *et al.*, 1994; Ramirez, 1994). The chemical senses, olfaction in particular, may contribute to fat sensation and preference (Larue, 1978; Ramirez, 1993b). Fat preference could be partly based on the detection of chemical impurities or decomposition products in fats (Ramirez, 1992). Since the present study used mineral oil rather than a nutritive oil, it is likely that the rats in these experiments responded to textural rather than to olfactory or gustatory cues. It is then noteworthy that, on the basis of the textural cues, deprived rats preferred a mineral oil emulsion over a 0.2% saccharin solution, a stimulus long recognized to be palatable to rats. Mineral oil appears to mimic imperfectly the orosensory profile of nutritive oils. This is indicated by the findings that rats prefer corn oil over mineral oil even when post-ingestive feedback is minimized (Ackroff *et al.*, 1990; Mindell *et al.*, 1990). Also, our preliminary work indicated that a relatively high (10%) concentration of mineral oil was needed to match the preference for 0.9% corn oil, a stimulus used in a previous study from our laboratory (Sclafani and Ackroff, 1993). Interestingly, in this prior study, food deprived rats preferred the 0.9% corn oil over 2% sucrose even though both nutrients were presented as suspensions stabilized with xanthan gum. In this case, viscosity probably differed little between the oil and sugar suspensions (Ramirez, 1992) and was not likely to play a role in the preference shift. Rather, other orosensory characteristics of the corn oil suspension were presumably responsible for the preference response of the deprived animals. Overall, it appears that fat sensation and preference are based on a multidimensional orosensory profile which remains to be fully identified and deprivation might alter the responses to several of its components.

Experiment 2 revealed that in their very first exposure to these stimuli deprived and sated animals showed similar preferences for mineral oil. However, following one-bottle exposure, mineral oil preference increased in the deprived rats but decreased in the replete animals. Thus, preference changed with experience.

Experiment 3 used a flavor conditioning paradigm to determine if deprivation state differentially affects the reinforcement value of saccharin and mineral oil. Both the saccharin-trained and mineral oil-trained groups displayed preferences for a flavor consumed while sated over a flavor consumed while hungry. These results, while interesting given prior claims that hunger selectively reduces the reinforcing effect of sweet taste (Capaldi, 1993), offer no insight into the experiential effect observed in the second experiment.

Why the deprivation-induced shift in preference is not seen in naive rats, but is seen after one-bottle exposure, remains to be explained. It is possible that rats are more neophobic to saccharin, which may have a complex (sweet/bitter) taste (Dess, 1993), than to mineral oil which may have a more bland flavor. During one-bottle exposure to saccharin, the non-deprived rats would lose their neophobia and subsequently be able to express their preference for saccharin. The deprived rats would also lose their saccharin neophobia during one-bottle exposure. However, since they could express their 'true' preference for mineral oil from the beginning, one-bottle experience would have less impact on their preference.

Whatever the reason for the one-bottle exposure effect, it did not permanently affect the rats' preference profile; reversing the rats' deprivation state altered their preference for saccharin and mineral oil. The preference reversal was not immediate, however, but took several days to develop. In particular, neither a single large meal immediately before the test (Experiment 1) nor 24 h of *ad libitum* feeding (Experiment 2) reversed the deprived rats' preference for mineral oil over saccharin. After 6 days on their new feeding schedule, with (Experiment 2) or without (Experiment 1) intervening preference tests, the NEW DEP groups preferred mineral oil and the NEW AL groups were either indifferent or preferred saccharin. These results indicate that flavor preferences are influenced by physiological signals related to long-term energy balance. Relevant to this point, Mook and Cseh (1981) reported that maintaining rats at different body weight levels by forced under- and overfeeding dramatically altered their intake of saccharin and dilute sugar solutions. Most interestingly, saccharin intake increased as body weight levels were reduced. This might appear to conflict with the present results, but Mook and Cseh (1981) only measured saccharin acceptance in one-bottle tests; they did not measure saccharin preference relative to oil or any other substance. The one-bottle data of the present experiment suggests that mineral oil intake may increase even more than saccharin intake as body weight decreases.

The effects of food deprivation on fat preference can be compared to those of another dietary manipulation: high-fat

feeding. Relative to rats fed a low-fat, high-carbohydrate diet, rats fed a high-fat, low-carbohydrate diet show increased acceptance of and preference for a variety of fats (Reed and Friedman, 1990; Reed *et al.*, 1990, 1991; Warwick *et al.*, 1990). This maintenance diet effect appears to be due, at least in part, to changes in the rat's responsiveness to the flavor of fat (Reed *et al.*, 1990; Reed and Friedman, 1990). Reed *et al.* (1991) proposed that the enhanced fat appetite displayed by rats fed a high-fat diet was due to their increased fatty acid oxidation. Since food deprivation also increases fatty acid oxidation (Mayes and Felts, 1967; McGarry *et al.*, 1973), it may be that a greater reliance on fat as a fuel played a role in the mineral oil preference displayed by the deprived rats in the present study. Note, however, that the rats switched from *ad libitum* to restricted feeding did not increase their mineral oil preference until several days after the dietary change; yet food deprivation stimulates fatty acid oxidation within 24 h (McGarry *et al.*, 1973). Thus, the role of fatty acid oxidation in deprivation-induced fat appetite is open to question.

In conclusion, food deprivation and/or weight loss alters the relative acceptance and preference for nutrient-related flavors. Choice tests with calorically dilute sugar and corn oil suspensions (Sclafani and Ackroff, 1993), and with non-nutritive saccharin and mineral oil solutions/emulsions (present study) demonstrate that food deprivation enhances oily preference and reduces sweet preference. Compared with controls fed *ad libitum*, food-deprived rats also show a greater preference for the oily flavor over the flavor of polycose, a non-sweet carbohydrate (Sclafani and Ackroff, 1993). These preference shifts are consistent with observations made with 'real' foods. That is, numerous studies have shown that in rats self-selecting their diet from separate macronutrient sources, food deprivation produces an increase in fat selection (Schutz and Pilgrim, 1954; Andik *et al.*, 1971; Piquard *et al.*, 1978; Reed *et al.*, 1988; Tempel *et al.*, 1989; Bligh *et al.*, 1990; Gerardo-Gettens *et al.*, 1991; Bernardini *et al.*, 1993). The present data indicate that deprivation could affect food selection in part by altering the immediate orosensory response. However, it is well documented that rats modify their preferences after experiencing the post-ingestive effects of foods (Sclafani, 1990). How deprivation interacts with preferences conditioned by different nutrients, such as fat versus carbohydrate, remains to be investigated.

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