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Food-conditioned odour rejection in the late stages of the meal, mediating learnt control of meal volume by aftereffects of food consumption

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In a two-bottle choice test, rats drank more of the fluid having a novel odour than that having an odour which had previously been presented in the later part of meals on concentrated maltodextrin solution. Rats are normally more averse to a novel odour than to a familiar odour; therefore, the conditioned reaction to the odour acquired in these circumstances is likely to be an ingestive aversion, rather than merely a lack of preference. Furthermore, this learnt odour rejection was seen only in the second half of the meal, indicating that it is dependent on an ingestion-induced state of repletion. Together then, these observations are evidence that the volume of meals rich in carbohydrate can be controlled by learnt rejection of particular food flavours in the presence of visceral cues specific to repletion (previously dubbed “conditioned satiety”), the only known mechanism by which aftereffects of ingested energy could reduce meal volume.

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Introduction

It has been proposed that much of the control of ingestion involves conditioned responses to conjunctions of external stimuli, such as the sensory characteristics of the foodstuff, and internal stimuli that depend on the current bodily state (Booth, 1977a, 1977b, 1985; Booth & Davis, 1973; Booth & Toase, 1983; Booth *et al.*, 1994; Booth *et al.*, 1976). That is to say, appetite and satiety are, at least in part, learnt preferences and aversions that vary with visceral state as it changes during a meal. Evidence in support of

this theory has accumulated over recent years in rats (Baker *et al.*, 1987; Booth, 1972b, 1977b, 1980; Booth & Davis, 1973; Gibson & Booth, 1986, 1989; Scalfani, 1997), monkeys (Booth & Grinker, 1993), and young (Birch *et al.*, 1987, 1990) and adult (Booth *et al.*, 1976; Booth & Toase, 1983; Gibson *et al.*, 1995) human beings. In all the cited experiments, there was evidence that the oral-visceral cue combinations were associatively conditioned by effects of an ingested foodstuff.

The first studies demonstrated that the volume of a meal on a distinctively flavoured diet could be controlled by aftereffects of a high concentration of the polymeric carbohydrates, dextrin or maltodextrin (MD), in the diet during prior training (Booth, 1972b; Booth & Davis, 1973). The volume of the meal on concentrated MD became smaller than the meal size for dilute MD, although the rates of intake at the start of the meals were similar: thus, the smaller meal on concentrated MD was not attributable to a flavour aversion from the beginning of the meal, as is produced by concentrated sugars (Booth *et al.*, 1972; Davis & Smith, 1990; Davis *et al.*, 1993; Warwick & Weingarten, 1996).

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Some of these effects on meal size might in principle have been a result of gastric adaptation to repeated liquid meals, rather than conditioning by the aftereffects of MD. However, there was a similar differentiation of meal size without a difference in initial rate in the first extinction test, i.e. on meals differing for the first time in only the flavour that had been paired with either high or low MD concentrations during training (Booth, 1972b). This showed that the reduction in intake was at least partly a learnt response to orosensory factors in the presence of an internal effect of eating much of a meal—presumed to be chemically non-specific distension of the digestive tract (an hypothesis subsequently confirmed by Gibson and Booth, 1989). Booth and Davis (1973) went on to demonstrate the specificity of lowered acceptance to the end of the meal by showing a reversal in two-choice preference from the start to the end of the meal after conditioning by concentrated MD. This learnt repletion-dependent flavour rejection was dubbed “conditioned satiety” to distinguish it from the conditioned aversion (and hypophagia) from the start of a meal seen with hypertonic sugars (Booth *et al.*, 1972; Davis & Smith, 1990; Le Magnen, 1959), hypoglycaemia (Le Magnen, 1957; Lovett & Booth, 1970) and amphetamine (D’Mello *et al.*, 1977; Le Magnen, 1963).

In a later experiment, Booth (1980) showed more directly that this learnt control of ingestion reflected a switch in relative preferences and aversions for the flavours during the meal after learning from postprandial aftereffects of concentrated and dilute MD. This was achieved by means of repeated two-stimulus choice tests, rather than merely dissociating meal size from initial rate or from two-choice preference as Booth (1972b) and Booth and Davis (1973) had done. After training, rats were given repeatedly enforced renewal of choices between a flavour previously paired with 40% MD and a 10% MD-paired flavour, in a test solution of intermediate MD concentration (i.e., extinction of differential aftereffects). Relative preference for the 40% MD-paired flavour at the start of the meal changed to a relative preference for the 10% MD-paired flavour at the end of the meal. The inference was that the changing preferences/aversions seen during extinction had been learned by the association of the aftereffects of either 10% or 40% MD with successive combinations of internal and external cues arising during the training meal on the flavoured MD.

However, as pointed out by Booth (1977a) and elaborated by Swithers Mulvey and Hall (1992; Swithers *et al.*, 1998), a reversal of relative preference during a meal might also arise from effects of habituation on the initial preference for one flavour, or through a simple alternation or reversal strategy, as shown by a rat exploring alternate arms in a maze. Evidence against this

was obtained from extinction tests in a second group of rats (Booth, 1977a) which were shown to maintain an initial conditioned preference (of similar strength to that for 40% over 10%) for a 25% MD-paired flavour over a 0% MD-paired flavour throughout the test meal: that is, this degree of conditioned preference did not habituate even towards the end of the meal, and no reversal of flavour choice was observed because 25% MD is not concentrated enough to condition aversively.

Booth (1977a) argued against habituation by comparing differently based preferences. The experiment reported here was designed to avoid any such habituation to the initially preferred odour, by restricting the presentation of odours to the second half of the test meal only, for the rats tested for a repletion-dependent odour rejection. In addition, another group of identically trained rats was given a choice of odours in the first half of the test meal only. This provided a conclusive between-subjects extinction test for the predicted lack of generalisation of the learnt odour rejection from the trained satiated state to the untrained hungry state, unconfounded by any alternation strategy. Problems of confounding by habituation and familiarization during the test were reduced by restricting exposure to the odour to 6 min for both groups.

The experiment was also designed to address the issue whether the odour which had been paired with aftereffects of concentrated MD is positively rejected or it is merely reduced in acceptance while the stomach is partly filled. Rats and other species reliably exhibit flavour neophobia (Revusky & Bedarf, 1967; Scott & Quint, 1946; Siegel, 1974), i.e. the animals are hesitant to consume novel foods, even when the first sampling has proved their flavour to be highly palatable. Also, olfaction appears to play a major role in this neophobic response (Hankins *et al.*, 1973). Therefore, if rats faced with consumption of either a familiar-smelling or a novel-smelling food late in the meal actually consume more of the novel-smelling food, this would indicate that the decrease in intake of the familiar-smelling food towards the end of the meal is likely to reflect rejection of the odour made familiar by training, rather than merely a relative preference for the untrained odour. We do not attempt to distinguish between aversion and avoidance or rejection, as others have done for learnt or unlearnt responses to tastes (Berridge, 1991; Pelchat *et al.*, 1983). In particular, this design neither requires nor allows assessment of hedonic or aversive reactions to the taste *per se* of the meals, and we know of no evidence that ingestive aversion to odours, which might involve more distal mechanisms, depends on expression of aversive fixed action patterns (FAPs).

The likelihood of obtaining aversively conditioning postingestional effects from a voluntarily ingested food

material such as glucose polymers was increased in this experiment by first adapting the rats to taking large meals of dilute carbohydrate when mildly hungry. These meals contained a non-nutritive thickener so that the texture of the dilute carbohydrate was similar to that of the more viscous concentrated MD solution to be introduced for aversively conditioning trials. In this way, the rats were "tricked" into consuming large amounts of concentrated MD solution at the start of training.

Method

Subjects

The experimental subjects were naïve adult male hooded rats, bred in the departmental Animal Laboratory at the University of Birmingham. At the start of the experiment, their mean (*SD*) body weight was 368 (45) g. The animals were housed singly in suspended steel cages, with an automatic water spout protruding from the centre of the back of the cage. Room temperature was $21 \pm 1^\circ\text{C}$. Prior to the start of the experiment, the rats were adapted to a 12-h:12-h light-dark cycle (lights on at 0500h; lights off at 1700h) and maintained on standard laboratory chow pellets (Diet 41B, Pilsbury Ltd., Birmingham, U.K.) with water available at all times.

Materials

The experimental diets used during adaptation, training and testing were prepared from solutions of various concentrations of maltodextrin and sugars. These liquid foods allowed easy monitoring of intakes at frequent intervals, while minimising interruptions of the animals' feeding bouts. Moreover, sensory differences arising from variation in source and concentration of carbohydrate (CHO) are more easily disguised and the acceptability of taste and texture maintained in liquid diets than they are in solid diets.

A 60% CHO solution was used in training and testing: this consisted of 60 g of a low-glucose mixture of gluco-oligosaccharides (maltodextrin "Snowflake" No. 01913, CPC, Manchester) per 100 ml of final solution, dissolved in distilled water. This level of polymeric CHO is roughly equivalent to that found mostly as starch in the maintenance chow (54 g per 100 g of chow, manufacturer's data).

A 6% CHO solution was used in adaptation and training: this consisted of 3 g of d-glucose (BDH Chemicals, Poole, U.K.) and 3 g of sucrose (BDH) dissolved in distilled water to a final volume of 100 ml. To match the viscosity of the 60% CHO solution, 0.7 g of

non-nutritive thickening agent was added (7HF sodium carboxymethylcellulose, Hercules, London). This 6% CHO formulation contained the same percentage of mono- and disaccharides as did the 60% CHO (MD) solution (manufacturer's data), and could not be reliably discriminated from that solution in paired-comparison tests by human observers; that is, these solutions were closely matched in texture and sweetness. It should be noted in any case that, if rats detect any other attractive cue in glucose oligomers, this experiment is assessing an aversively conditioning effect of their consumption.

The odorants used as sensory cues to be conditioned were food essences of almond and violet (both Rayner, London). For 16 rats, the odour concentrations were 2% almond and 1% violet. However, these levels of odorants were subsequently considered to be unnecessarily intense, with a risk of inhibiting drinking, and so, for the remaining eight rats, the concentrations were 1% almond and 0.1% violet, with 0.25% vanilla essence (Pearce Duff, Dunstable) included with the violet to provide a similar "sweetish" aroma to that of the almond (for human observers). There was no evidence that this variation in odour cues systematically affected drinking across rats.

Adaptation

The rats were first accustomed to drinking from two 10-ml (0.1-ml calibrated) glass tubes attached to the front of their cages. On the first day, they had access overnight to two tubes of tap water, after the maintenance water spouts had been removed. On the second day, the rats were given access to two tubes containing 0.1% saccharin in distilled water for 2 h towards the end of the light phase. This procedure was repeated on the following day.

With drinking from the tubes firmly established, the rats were placed on a food-deprivation schedule in which maintenance chow was removed 4 h before the CHO meal and replaced 2 h later. First, they were adapted to meals of thickened 6% CHO (Table 1). This solution was presented in two tubes as above, during the second half of the light phase. The rats had 45 min access for the first 2 days, and thereafter access for 30 min, by the end of which period drinking had virtually ceased. On the third and fourth days of access to 6% CHO, the positions of the tubes were reversed three times at 5, 10, and 20 min after the start of the meal. From the fifth to the ninth day, tubes were reversed five times at the 2-, 4-, 8-, 12-, and 16-min intervals. This adapted the rats to the frequent tube reversals that would be made during conditioning and testing in order to monitor brief two-choice preference/aversion.

Table 1. Schedule for conditioning and testing for 60% CHO-paired odour (O+) aversion relative to novel odour (O-)

Experimental stage	Times of day and procedures		
<i>Adaptation</i> (9 days)		1400–1430h Access to 6% CHO with no odour	
<i>Training</i> Days 1 and 3	1400–1406h 60% CHO no odour	1406–1420h 60% CHO + odour	
Day 2		1400–1430h 6% CHO no odour	
<i>Odour-choice testing</i> Either (1st-half test)	1400–1406h 33% CHO O+ vs. O-	1406–1412h 60% CHO no odour	1412–1420h 60% CHO no odour
Or (2nd-half test)	33% CHO no odour	60% CHO O+ vs. O-	60% CHO no odour

Note: On all days, rats were deprived of chow at 1000h and re-fed at 1630h.

After 9 days of access to 6% CHO, intakes had stabilized and the rats were considered sufficiently adapted for training to begin.

Conditioning

The training procedure was similar to that used during adaptation (Table 1). After 4 h of food deprivation, the rats were presented with two 10-ml tubes containing unodourized 60% CHO. Each rat was first presented with each tube separately until it had approached and licked both tubes. These were then simultaneously clipped to the cage-front, and the initial volumes were noted. This “forced sampling” procedure was used every time two new tubes were offered, and was intended to increase sensitivity of the preference/aversion measure to odour-related choice. Reversal of the tube positions was designed to match the subsequent preference/aversion-testing procedure. Positions were reversed at 2, 4, and 5 min.

At 6 min (approximately the stage when half the volume of the meal had been consumed), all tubes were removed and immediately replaced by new tubes containing odourized 60% CHO: half the rats were trained with almond and half with violet odour. Tube positions were reversed again at 8, 10, 11, 12, and 16 min. Volumes were recorded at every solution change and position reversal. At 20 min, final volumes were noted and the tubes removed.

After the first training day, the rats underwent a further adaptation day: that is, they were given a meal of unodourized 6% CHO exactly as for the previous adaptation days. This was intended to increase the likelihood that conditioning was in fact differential between the aversively or appetitively reinforcing aftereffects of 60% and 6% CHO. That adaptation day was then followed by a second conditioning day, identical in procedure to the first.

Odour aversion testing

The position-reversal and tube-presentation procedure was the same as for training (Table 1). However, the rats were given a 33% CHO solution (a 1:1 mixture of the 60% and 6% CHO solutions) for the first 6 min. It was hoped that this solution would improve the chances, by the second half of the meal, of creating substantial gastrointestinal distension (the likely internal cue element for learnt meal-volume control). At 6 min, the 33% CHO solutions were replaced with 60% CHO. Sixteen rats (eight almond-trained, eight violet-trained) were given a choice between the 60% CHO-paired odour and a novel odour (i.e. always almond and violet odours) for the first 6 min of the meal (33% CHO), followed by unodourized 60% CHO for the second half of the meal. The remaining eight rats (balanced for trained odour) were given unodourized 33% CHO for the first 6 min, and then a choice between almond and violet in 60% CHO, for the next 6 min. At 12 min, the odourized 60% CHO was replaced with unodourized 60% CHO, for the remaining 8 min of the test meal. Again, volumes were recorded at every position reversal and solution change.

In this way, a measure of preference/aversion for the trained odour relative to a novel odour was obtained in some rats during the first half of the meal while they were hungry, and in the other rats in the second half while they were partly satiated. At the same time, odour exposure was equated between groups by restricting access to the odour-choice to 6 min.

Analysis of data

Preference/aversion ratios (intake of the trained odour over total intake) were calculated for the first meal-half or second meal-half 6-min odour choice tests. Within each of these choice tests, intake ratios free from

position bias could be obtained after one and two "position-balanced" periods. That is, these are periods over which a rat had an opportunity to drink the same total volume with the two odours positioned left and right as when right and left. These periods occurred after 4 and 6 min for the first meal-half tested group, and after 10 and 12 min for the second meal-half tested group. The mean preference/aversion ratios were compared to 0.50 (indifference) by related t-test. The first half and second half ratios, from equivalent position-balanced periods, were compared to each other by independent t-tests. The second-half ratio was predicted to be less than 0.50, and less than the first half ratio, and so one-tailed tests are appropriate for these comparisons.

For accuracy, preference/aversion ratios were calculated only for rats whose intake for that period exceeded 0.3 ml (5 kcal). This resulted in fewer rats being included in the analysis for the second half of the meal than in that for the first half. The results given here are from three groups of rats ($N = 7-11$) run through the above design on separate occasions. There was no evidence of any difference between runs, and so the data were combined for analysis.

Results

Concentrated CHO-induced odour rejection late in the meal

The preference/aversion ratios obtained from the first position-balanced period in the second half of the test meal (6–10 min) were significantly less than 0.50 (Fig. 1), $t(7) = 2.54$, $p < 0.025$, one-tailed, indicating a significant aversion to (or avoidance of) the 60% maltodextrin-paired odour relative to the novel odour. Ratios calculated from total intakes over the two position-balanced periods in the second half of the test meal (6–12 min) were also significantly less than 0.50 (Fig. 1), $t(7) = 1.96$, $p < 0.05$, one-tailed.

Dependence of the learnt rejection on the repleted state

The mean preference/aversion ratios from those rats tested in the first half of the meal were not different from 0.50, after either one or two position-balanced periods (Fig. 1), indicating that there was no learnt rejection before repletion had set in. Also, the mean preference/aversion ratio from the first position-balanced period in the first half of the meal (i.e. at 4 min) was significantly greater than the mean ratio (at 10 min) from rats tested in the second half of the

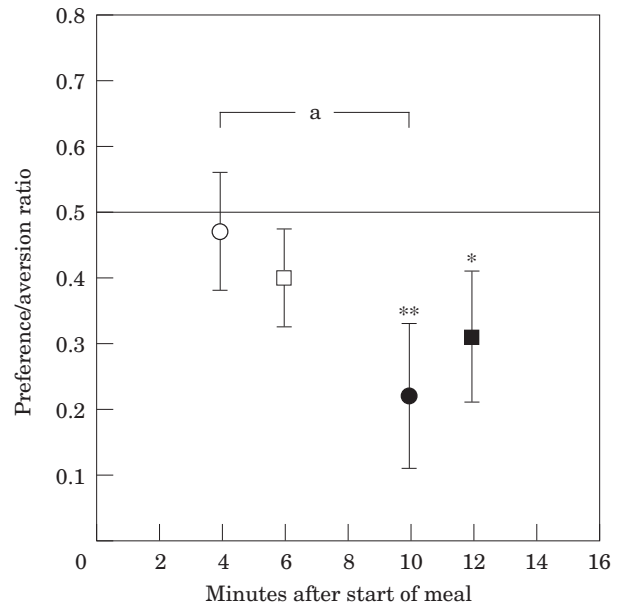


Figure 1. Preference/aversion ratios for the trained odour relative to the novel odour for rats tested either in the first half (open symbols; $N = 15$ or 16) or second half (filled symbols; $N = 8$) of the meal, after one (0–4 or 6–10 min; circles) and two (0–6 or 6–12 min; squares) position-balanced periods (i.e. periods when intake has been balanced for left-right position bias either once or twice; see the text for details). Preference/aversion ratio is calculated from intake of the trained odour divided by total intake of both odours for that period. Data are expressed as mean \pm SE. * $p < 0.05$; ** $p < 0.025$, one-tailed, preference/aversion ratio < 0.50 . ^a $p < 0.05$, one-tailed, 2nd-half ratio < 1 st-half ratio.

meal (Fig. 1), $t(21) = 1.79$, $p < 0.05$, one-tailed. This is evidence that the learnt rejection depends on the presence of a more nearly replete state, i.e. it is the learnt response to a combination of oral and visceral repletion cues that does not generalize to the combination of visceral depletion cues and the same odour.

Preference/aversion ratios calculated from intakes during two position-balanced periods (i.e. at 6 or 12 min) did not differ significantly between groups tested in the first and second halves of the test meal, although the mean values were in the predicted direction (Fig. 1). This is a less sensitive and indeed less valid test for the presence of a learnt aversion, in the sense of rejection in favour of a novel odour, since the longer period of access increases the likelihood of familiarization with the novel comparison odour.

Differences in intake

Intake varied over the three meal stages, i.e. 0–6 min, 6–12 min and 12–20 min (Fig. 2), meal stage main effect, Wilk's Lambda = 0.153, epsilon-corrected

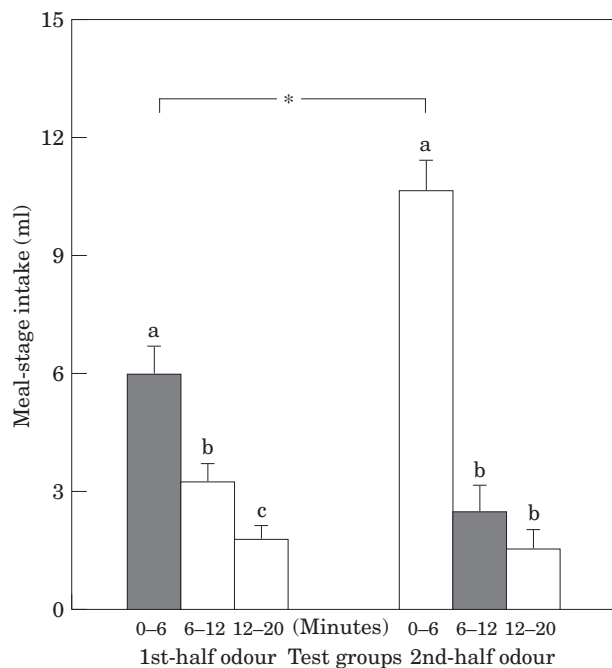


Figure 2. Test day intakes over the three meal stages for rats given the odour choice (trained vs. novel odour) either in the first 6 min period (0–6, 1st-half odour group) or second 6 min period (6–12, 2nd-half odour group). For all rats, the test solution was 33% CHO for 0–6 min, and 60% CHO for the remainder of the meal. Data are expressed as mean + SE. * $p < 0.01$ that 0–6 min intakes are equal between groups. Significant differences in intake between adjacent meal stages, within groups, are indicated by different letters: a vs. b, $p < 0.001$; b vs. c, $p < 0.05$. Odour choice, ■; No odours, □.

$F(2, 44) = 84.1$, $p < 0.001$, and meal-stage intake interacted with test group, i.e. first-half or second-half odour choice groups, epsilon-corrected $F(2, 44) = 15.0$, $p < 0.001$. However, overall intake for the entire meal did not differ significantly between first-half and second-half test groups, group main effect, $F(1, 22) = 3.95$, $p = 0.06$.

In the first half of the meal (0–6 min), rats given the odour choice at this stage ate less than the group given no odour choice then, $t(22) = 4.06$, $p < 0.01$, two-tailed (Fig. 2). It must be noted that any effect of odour in this analysis is collapsed across effects of both the novel odour and the trained odour, and that on average across the first-half odour-choice group, intake did not differ between these odours (see above). Subsequent intakes did not differ between groups over either 6–12 min or 12–20 min (Fig. 2).

Rats in both groups ate less during the 6–12 min stage than at 0–6 min: first-half odour-choice group,

$t(15) = 6.14$, $p < 0.001$; second-half odour-choice group, $t(7) = 6.18$, $p < 0.001$. Intake at 12–20 min was significantly less than at 6–12 min only for the group given the odour choice at 0–6 min, i.e. first-half tested: first-half odour-choice group, $t(15) = 2.66$, $p < 0.05$; second-half odour-choice group, $t(7) = 1.60$, *NS*.

Discussion

The odour paired with concentrated maltodextrin was rejected in the presence of a novel odour, which would normally be expected to be relatively aversive. If the trained odour was merely less liked or neutral, its rejection would require the novel odour to be positively attractive. This seems unlikely, although we have no direct evidence here of the direction of influence of the novel odour on ingestion. So, these results are the strongest evidence to date for an aversion to an almost certainly olfactory cue (Baker & Booth, 1989) that has been conditioned by food, presumably acting post-ingestively after it had been swallowed.

Berridge (1991) has argued that true aversion to tastes (i.e. actual dislike rather than relative avoidance due to less liking) should elicit gaping, repellent tongue movements and other aversive FAPs, whereas satiation (sensory or caloric) reduced ingestive actions but did not increase aversive actions. We have not measured such responses here, but our design demonstrates that the rats have learnt to avoid the odour, not the taste or texture of the solution. These latter two sensory properties did not differ between the two bottles in the choice test, and furthermore the rats were exposed to similarly tasting but dilute (not strongly satiating) CHO meals before and between odour training days. Therefore, we have no reason to expect aversive FAPs to the taste here, and there is no evidence that revolting odours necessarily elicit the gapes that bitter tastants and toxin-paired tastes do. It is an open question whether other “aversive” behaviours were elicited by the rejected odour, and whether this odour rejection would differ qualitatively from toxin-paired odour aversion, but the replete animals clearly consumed less of the trained odour than of the novel.

The results are also evidence that this learnt rejection of the odour depends on the presence of cues from a state of repletion brought about by a substantial intake of fluid. That is, as hypothesized, rejection of the odour did not generalize from the trained replete state to the implicitly untrained depleted state in the first part of the meal. This is consistent with learning not to ingest food having a certain odour in the presence of a specific visceral cue when the oronasal cues had been paired with aftereffects of ingesting concentrated maltodextrin.

The presence of repletion during this second meal-half choice test is supported by the evidence of considerable slowing of intake at that stage. The odour-dependent inhibition of intake during the first half of the meal likely reflects some non-selective suppressant effect of the unexpected presence of odours at the start of the meal. In any case, in contrast to later in the meal, intake at this stage did not differ between trained and novel odours, whatever motivational valence each odour may have had at that moment. Although the odour choice during the first half of the meal was presented in 33% CHO, this was unlikely to have confounded any learnt response to the odour, as the solution's taste and texture were similar to both the 6% and 60% CHO, whose odourless qualities could not predict state-dependent postingestive effects given the present schedule.

This evidence of meal-end loss of acceptance was not confounded by habituation, familiarisation, or some preference-switching tendency, as earlier experiments may have been. So, it provides a strict test of the theory that part of normal meal-volume control is a learnt repletion-dependent rejection of the sensory characteristics of particular foods (Booth, 1972b, 1977b, 1980, 1985). The findings strongly support this interpretation of earlier, behaviourally less conclusive data. Thus, the volume of a meal is not determined just by the decrease in all palatability as deprivation arousal declines (Booth, 1972a; Cabanac, 1971) and the decrease in the palatability specifically of the food just eaten (e.g. Berridge, 1991; Booth, 1976; Clifton *et al.*, 1987; Rolls *et al.*, 1983). Termination of eating is also a learnt decrease in the facilitation of ingestion that is specific to visceral cues which have been configured with dietary cues by pairing with a prompt and mildly aversive aftereffect of concentrated maltodextrin (Booth & Davis, 1973), e.g. osmotic distension of the duodenum by rapid digestion of the oligomers in food eaten at the start of the meal that were dumped from the stomach before its emptying became controlled (Hunt, 1983). Indeed, this conditioning mechanism provides some control of a meal's volume by its usual energy contents, unlike the habituation of sensory preference or the gating of palatability by gastric distension.

It is worth noting that the four preference/aversion ratios obtained here over the course of the meal (Fig. 1) form a triangular pattern reminiscent of that seen in human ratings of learnt appetite and preference, where the apex represents the ideal sensory stimulation in that sensory, somatic and social context, and the sides are decrements in desire with deviation from ideal (Booth, 1986; Booth *et al.*, 1994). For learnt aversions, the triangle is inverted, and its apex, then a nadir, i.e. the strongest rejection, should be found at the stage in the meal at which the visceral cues during testing are most

similar to those of the trained configuration of visceral state with odour. At least part of the reason for the strongest rejection at 10 min rather than 12 min may be that 33% CHO was used rather than 60% CHO for the first half of the test meal, in order to encourage rapid and substantial upper-gut distension by the second half: thus, the learnt distension cue would occur earlier during testing than it did during training, generating the premature peak rejection of Fig. 1.

Evidence for conditioned satiety, such as in this experiment, has so far only been obtained using concentrated solutions of mixtures of glucose oligomers (MDs), albeit voluntarily ingested and within the normal range of concentrations of starch in food. Hypertonic glucose solutions are not suitable for use in experiments on conditioned satiety because they have osmotic effects in the gut that can condition state-independent aversions (Booth, 1972b, 1979; Booth *et al.*, 1972; Davis *et al.*, 1978; Shuford, 1959). Hence results from 0.8 M sucrose, for example, are not relevant to the phenomenon of aversion conditioning specific to the ends of meals, contrary to Davis and Smith (1990). In a similar fashion, the finding of Warwick and Weingarten (1996) that flavours paired with ingestion of 24% and 30% sucrose were consumed less than flavours paired with sham feeding or 5% sucrose, respectively, may reflect such aversive osmotic effects, confounding interpretation in terms of conditioned satiety. These state-independent sensory aversions induced by aftereffects of strong sugar solutions have more relevance to the avoidance of toxins (Garcia *et al.*, 1985) than to the normal control of meal volume. Such unpalatability at the start of eating could not control the volume of meals on a choice of foodstuffs. In any case, the undeniable fact is that many foods are attractive when hungry and so conditioned aversions affecting early consummatory or ingestive behaviour must be irrelevant to the normal control of intake.

Therefore, in any demonstration of conditioned satiety, evidence for state dependency is crucial. Ingestive aversion to food in the presence of a consumption-induced repletion state differentiates the rejection of food cues in any motivational state including hunger, which would be a general ingestive aversion, from the rejection of food cues specifically towards the end of a meal, i.e. satiated cessation of eating. This is still a sensory aversion, but a state-dependent one. Thus, a reduction in meal size elicited by a CS in a single-stimulus test, even if nutrient-conditioned (Le Magnen, 1955, 1959), is not sufficient evidence for conditioned satiety (Booth, 1972b).

The partitioning of meal stages in the design used here allowed a clear demonstration of the rats' changing evaluation of the odour previously paired with

concentrated MD during a single meal. Previous attempts from other laboratories to study the impact of repletion on conditioned responses to ingested flavours have tested preferences for flavours paired with real- and sham-fed liquid meals, or compared preference/aversion ratios for flavours paired with infusions of different concentrations of MD (Lucas *et al.*, 1997; Sclafani *et al.*, 1994; Van Vort & Smith, 1983; Warwick & Weingarten, 1996). However, these groups measured only meal sizes or longer-term intakes, and so early depletion-specific responses were not assessed for comparison with later meal changes. Thus, changes in relative preference/aversions from those designs may have been confounded by habituation, flavour-flavour conditioning, state-independent aversions and/or unconditioned reactions to innately palatable flavours.

The results of this study fully substantiate the long-standing refutation (Booth, 1972b; Booth & Davis, 1973) of the traditional theory in physiology that meal size is determined by innate visceral inhibition subtracting from a constant orosensory facilitation (Davis *et al.*, 1976; Stellar, 1955). Nevertheless, that theory is still revived on the basis of methodologically dubious data, e.g. effects of non-absorbable additives (Davis *et al.*, 1976) or hypertonic sugar solutions (Davis & Smith, 1990; Davis *et al.*, 1993), and ratings of "pleasantness" or "palatability" arbitrarily interpreted by investigators who have not measured the controlling cues (Rogers, 1999; Yeomans, 1996). Instead, the relationship between sensory preferences and intake needs to be studied by measuring the influences of specified momentary stimulation on symbolic or concrete measures of appetite (Booth *et al.*, 1986; Gibson & Booth, 1987).

In conclusion, these findings provide evidence for the only mechanism proposed so far for the control of meal volume by aftereffects of ingested energy. This learnt link between amount of food eaten and the aftereffects of eating has recently become acknowledged again as the explanation of increased energy intake on high-fat or energy-dense diets (Blundell *et al.*, 1995; Poppitt & Prentice, 1996; Stubbs *et al.*, 1998). Clearly, such caloric over-consumption is far from passive when an unfamiliar increase in the energy content of meals results in increased intake of energy by active performance of the learnt control of volume eaten.

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