ROLE OF PORTAL AND PLASMA GLUCOSE ELEVATIONS IN TASTE-TO-POSTINGESTIVE CONSEQUENCE LEARNING

By

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A Thesis
Submitted to the School of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree
Doctor of Philosophy

McMaster University

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TASTE-TO-POSTINGESTIVE CONSEQUENCE LEARNING

DOCTOR OF PHILOSOPHY (1992)
(Psychology)

MCMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Role of Portal and Plasma Glucose Elevations in Taste-to-Postingestive Consequence Learning

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NUMBER OF PAGES: xi, 111

ABSTRACT

Animals are able to associate the taste of a food with its positive nutritive consequences and will modify their feeding behavior when re-exposed to tastes on the basis of this association. However, the specific nutritive consequence(s) that animals detect and associate with a food's taste are unknown.

This thesis tested the ability of two postingestive events, elevations of plasma or portal glucose, to support taste-to-postingestive consequence learning.

Two strategies were used to evaluate the relevance of elevations in plasma glucose in taste-to-postingestive consequence learning. First, since real, but not sham, feeding supports taste-to-postingestive consequence learning, I recorded plasma glucose levels during sham and real feeding (Experiment 1). For the first 15 minutes of the meal, both sham and real feeding produced similar elevations in plasma glucose. Thereafter, real feeding produced sustained elevations in plasma gluocse, whereas sham feeding did not. Next, I paired tastes during sham feeding with a glucose infusion into the jugular vein, calibrated (Experiment 2) to mimick the plasma glucose profile of real feeding. Since rats that experienced tastes paired with plasma glucose elevations characteristic of real

feeding, did not demonstrate taste-to-postingestive consequence learning (Experiment 3), plasma glucose elevations must not support taste-to-postingestive consequence learning. In Experiment 4 I also demonstrated that sham feeding produces elevations in plasma glucose because nutrient is absorbed.

To assess the relevance of portal elevations in taste-to-postingestive consequence learning, I paired flavoured saccharin or flavoured glucose solutions with glucose infusions in the portal vein (Experiments 6 & 7). Only rats that received portal infusions of glucose paired with flavoured glucose solutions demonstrated taste-to-postingestive consequence learning (Experiment 7). Since oral glucose solutions, alone, elevate glucose levels in the portal vein, portal elevations of glucose may only support taste-to-postingestive consequence learning at suprathreshold levels. Alternatively, signal(s) arising from the stomach or gastrointestinal tract may potentiate the relevance of elevations of portal glucose since oral glucose solutions also stimulate these sites.

ACKNOWLEDGEMENTS

I thank Harvey P. Weingarten, my supervisor, for the countless hours he spent with me, planning and discussing the experiments in this thesis. These lively discussions kept me challenged and will shape my scientific thinking for years to come.

Next, I thank my committee members, Ron Racine and Shep Siegel for helpful comments on earlier drafts of this manuscript. I also thank them for allowing me to complete an applied internship at Monell Chemical Senses Center in the summer of '91. I was aptly supervised at Monell by Michael G. Tordoff and completed Experiments 6 & 7 during my stay there.

I thank other faculty members of the Psychology department who willingly engaged in "hall" conversations about research and teaching. Specifically, I thank Paula Durlach, Jeff Galef, Steve Tipper and Martin Daly in this regard.

Many hours were spent during my years at Mac in room 319 and the surrounds. My time there was made enjoyable by current and former labmates in the Weingarten lab. The list of Weingarten labmates is long and includes Bill Parkinson, Richard Black, Michael Bédard, Vicki Smye, Dawn Elston, Heather Looy, Fiona Ghosh, Anh Duong, Kevin McHugh, "Whit"

Whitten and Siobhan Callahan. My classmates, in particular Sheree Kwongsee, also added to my life at Mac. In the final hours, Peter Lind saved me from the wrath of graduate studies by formatting this thesis. Thank you Peter!

Last, but not least, I am grateful to teachers, friends and family who, over the years, encouraged me to learn new things. NSERC and a Heinz Foundation Scholarship at Monell provided the means to return to school.

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CHAPTER 1. GENERAL INTRODUCTION

The ability of animals to develop aversions to tastes that produce illness is well known (Riley & Tuck, 1985). But, animals can also learn to prefer tastes that have been associated with the positive, nutritive consequences of food. Many have shown that animals' feeding behavior with a taste depends, in part, on that taste's previous nutritive consequences (for reviews see Booth, 1985; Rozin & Zellner, 1985; Sclafani, 1990, 1991; Tordoff, 1991).

Animals' ability to form positive taste-to-postingestive associations has been called "taste-to-postingestive consequence learning" (Weingarten & Kulikovsky, 1989), "flavor-nutrient conditioning" (Sclafani, 1991), "conditioned satiety" (Booth, 1972) and "calorie-based learning" (Mehiel & Bolles, 1984; Tordoff, Ulrich & Sandler, 1990). For the purposes of this thesis, this phenomenon will be described as "taste-to-postingestive consequence learning" where "taste" is defined in its broadest sense: that is, as the oral and olfactory stimulation produced by food in the mouth.

Two behavioral probes have been used to index taste-to-postingestive consequence learning. The first is the establishment of a *taste preference* for tastes previously paired with nutritive postingestive consequences. The second is a *change in meal size* based on a taste's previous postingestive consequences. These behavioral probes will be described in turn.

Learned Taste Preferences

Animals can develop a preference for a taste based on its previous beneficial postingestive consequence(s). The taste of a food is naturally paired with its postingestive consequences during eating. Thus, eating provides an opportunity for animals to develop taste preferences that can then guide food selection. In the laboratory, this phenomenon is mimicked by pairing one arbitrary taste (the conditioned stimulus, CS+) with a nutritive postingestive consequence eg. polycose solutions (the unconditioned stimulus, UCS), and another taste (the CS-) with a nonnutritive postingestive consequence eg. water or saline. Under such laboratory conditions, animals routinely prefer tastes that have been paired with nutritive postingestive consequences over tastes that have been paired with nonnutritive consequences (for reviews see Sclafani, 1990, 1991; Tordoff 1991).

Taste preferences produced by taste-to-postingestive consequence learning can be computed in a variety of ways. Frequently, CS+ intake is simply compared to CS- intake: a taste preference is said to be present if CS+ intake is greater than CS- intake. However, taste preferences can also be assessed by comparing the <u>number</u> of subjects who demonstrate a taste preference (CS+ intake > CS- intake), to the <u>total number</u> of subjects.

Regardless of the computational technique used to assess taste preferences, subjects' preferences should be assessed during simultaneous, rather than sequential, access to the CS+ and CS- (for a discussion of the superiority of simultaneous [eg. 2-bottle preference tests] vs sequential access [eg 1-bottle preference tests] see Weingarten & Bedard, 1990.)

A) Oral Conditioning

The term "oral conditioning" has been used to describe the establishment of taste preferences for tastes paired with the oral ingestion of nutrient (eg. Sclafani, 1990, 1991). Taste preferences produced by food ingested orally, typically add distinctive flavours to a nutritive solution or food that rats then ingest. Taste preferences can also be produced if distinctively flavoured non-nutritive solutions are presented in separate containers with nutritive solutions or food.

Rats' ability to develop taste preferences through oral conditioning is independent of the macronutrient chosen to provide the postingestive consequence(s). Rats can develop taste preferences for tastes paired with nutritive solutions of alcohol (Mehiel & Bolles, 1984), sugar (Messier & White, 1984) or starch (Elizalde & Sclafani, 1988). Taste preferences can also be established by pairing tastes with nutritive fat emulsions (Elizalde & Sclafani, 1990; Tordoff, Tepper & Friedman, 1987).

Although some studies have produced taste preferences by giving rats extensive experience with tastes and their postingestive consequences (eg. Hamilton, 1964; Mehiel & Bolles, 1988; Messier & White, 1984), brief exposure to tastes and their postingestive consequences is sufficient to establish taste preferences. Food-deprived rats given two pairs of thirty minute trials with flavoured saccharin alone and flavoured saccharin plus corn oil, displayed a 94% preference for the flavour of saccharin paired with the nutritive corn oil emulsion (Elizalde & Sclafani, 1990).

Establishing taste preferences with postingestive consequences ingested orally is straightforward, noninvasive and mimics the conditions of normal eating. However, taste preferences produced by oral conditioning can be difficult to interpret. Taste preferences established by oral conditioning may reflect taste-to-postingestive consequence

learning but they may also reflect flavour-flavour learning. Rats that experience an arbitrary taste or flavour (CS+) paired with a nutrient may form an association between the CS+ and the nutrient's taste (flavour-flavour learning) as well as, or instead of, an association between the CS+ and the nutrient's postingestive consequences (taste-to-postingestive consequence learning).

The suggestion that flavour-flavour learning confounds taste preferences produced by oral conditioning is reinforced by demonstrations that non-nutritive substances such as mineral oil emulsions (Elizalde & Sclafani, 1990) or saccharin solutions (Holman, 1975; Messier & White, 1984) can support taste preferences when paired with non-nutritive tastes.

Elizalde & Sclafani (1988) suggested that flavour-flavour conditioning occurs only if there is no, or little, delay between the conditioned taste stimulus (CS+) and the nutritive taste (UCS) while taste-to-postingestive consequence learning can occur whether or not a delay is imposed between the CS and the UCS. Their suggestion is based on the demonstration that saccharin, a nonnutritive sweet taste, only supports a flavour preference when it immediately follows the CS+ while glucose, a nutritive sweet taste, can support a taste preference whether it immediately follows the CS+ or is delayed by thirty minutes (Holman, 1975).

While taste preferences produced with CS-UCS delays during oral conditioning may be unconfounded by flavour-flavour learning, not all attempts to produce preferences under these conditions have been successful. For example, taste preferences have been produced when a CS+ flavour is followed ten to thirty minutes by glucose (Holman, 1975; Simbayi, Boakes & Burton, 1986), polycose (Capaldi, Campbell, Sheffer, & Bradford, 1987) and fat emulsions (Elizalde & Sclafani, 1990), but similar CS-UCS delays have also failed to produce taste preferences (Simabyi et al, 1986; Elizalde & Sclafani, 1988).

One of the problems with imposing a CS-UCS delay in oral conditioning is that a CS-UCS delay places the taste of the UCS in much closer temporal proximity to the postingestive consequences than the CS+ flavour. Thus, the taste of the UCS, and not the CS+ flavour, may become associated with the nutrient's postingestive consequences. Perhaps this is why some studies fail to produce taste preferences during CS-UCS delays.

There is evidence that oral conditioning can be facilitated during a CS-UCS delay if rats have previously learned that a UCS (and its taste) is nonnutritive. Elizalde & Sclafani (1988) pre-exposed one group of rats to a polycose solution that contained acarbose, a drug which inhibits absorption by inhibiting the digestion of polycose; a second group of rats received no pre-exposure experience.

Subsequently, all rats entered the conditioning phase of the experiment where flavoured saccharin solutions were followed, after a ten minute delay, by an unadulterated polycose solution or nothing. Both groups showed a taste preference for the CS+ flavour paired with polycose but the preference was stronger (69% vs 53%), and required fewer conditioning trials (2 pairs vs 4 pairs of trials), in the group that had been pre-exposed to polycose + acarbose. This study suggests that learning the taste of the UCS was nonnutritive allowed rats to associate the "novel" postingestive consequences of polycose with the only novel flavour that preceded it: namely, the CS+. This study also suggests that acarbose eliminates or attenuates a relevant consequence in taste-to-postingestive consequence learning.

The ability of pre-exposure to a polycose + acarbose solution to facilitate oral conditioning during CS-UCS delays has been used to test the temporal constraints of taste-to-postingestive consequence learning. Elizalde & Sclafani (1988) demonstrated that rats pre-exposed to a polycose + acarbose solution subsequently formed a taste preference for a flavour followed 60 minutes later by unadulterated polycose. Producing taste-to-postingestive consequence learning with a sixty minute CS-UCS delay is impressive and suggests that, like taste aversion learning, taste-to-postingestive consequence learning can occur when there is considerable delay between the CS and the UCS.

This provides additional evidence that taste-to-postingestive consequence learning occurs during normal eating since the postingestive consequences of eating can occur minutes to hours after food has been ingested.

B) Gastric or Duodenal Conditioning

The term "gastric or duodenal conditioning" refers to the establishment of taste preferences for tastes paired with the gastric or duodenal delivery of nutrient. Taste preferences produced by nutrient delivered to the stomach or duodenum are easier to interpret than preferences produced by oral conditioning. In oral conditioning, the nutrient's taste can contribute to a taste preference through flavour-flavour learning. When nutrient is delivered directly to any site beyond the mouth, the nutrient's taste cannot confound conditioning.

Various techniques permit nutrient to be delivered directly to the stomach or duodenum. Gavaging nutrient into the stomach is the least invasive technique and can produce taste preferences (Tordoff, Ulrich & Sandler, 1990) but it also disrupts ongoing eating. Therefore, nutrient is more commonly delivered via chronic indwelling catheters in the stomach or duodenum.

Taste preferences have been produced by intragastric (IG) infusions of glucose (Sherman, Hiskis, Rice, Rusiniak, & Garcia, 1983), polycose (Elizalde & Sclafani, 1988) and

fat (Lucas & Sclafani, 1989). But not all infusion studies have produced taste preferences and some have even produced taste aversions (eg. Deutsch, Molina & Puerto, 1976; Ramirez, 1984).

Studies that fail to produce a taste preference with gastric or duodenal infusions do not necessarily imply that it is difficult to form taste-to-postingestive consequence associations. In infusion studies, the concentration of the infusate, as well as the infusion rate and volume are arbitrarily defined by the experimenter. These arbitrary parameters may produce malaise, a state that rats readily associate with a food's taste. Malaise-induced aversions may then offset taste preferences produced by the taste's nutritive consequences. Taste preferences may also be offset by taste aversions produced by administering undigested nutrient directly to the stomach or duodenum. In normal ingestion, food enters the mouth and is partially digested by oral enzymes. Nutrient that bypass the mouth, enters the gastrointestinal tract undigested and can be aversive. For example, rats preferred flavours paired with intragastric infusion of saline or nothing to flavours paired with intragastric infusions of undigested milk (Puerto, Deutsch, Molina, & Roll, 1976) while intragastric infusions of milk "pre-digested" by donor rats produced a taste preference (Puerto et al, 1976).

The "arbitrariness" of infusion parameters can be reduced with the electronic esophagus preparation developed by Mook (1963). In this preparation, rats control the rate of gastric infusions by their own oral ingestion; gastric infusion rates approximate rates of oral intake and are determined on an individual animal basis. When this preparation is used to condition a taste preference, rats are given 23 hour access to flavoured non-nutritive solutions paired with intragastric infusions of nutrient or water, one condition/day. This protocol has established flavour preferences with carbohydrate (Elizalde & Sclafani, 1988) or fat infusions (Lucas & Sclafani, 1989) as the nutritive consequence.

Preferences produced with an electronic esophagus preparation can be striking and robust. For example, after four pairs of conditioning trials, rats displayed a 90% preference for the flavour that had been paired with an IG infusion of polycose (Elizalde & Sclafani, 1990). Rats retained this strong preference when tested after two weeks of extinction training where CS+ intake was paired with intragastric infusions of water (Elizalde & Sclafani, 1990). But flavour preferences established with an electronic esophagus preparation are not insensitive to reinforcement contingencies; flavour preferences reverse when reinforcement contingencies are reversed. Rats with a strong CS+ preference, developed a preference for the CS-

flavour when the former CS- flavour was paired with intragastric infusions of polycose and the former CS+ flavour was paired with intragastric infusions of water (Elizalde & Sclafani, 1990).

C) Intravenous Conditioning

The term "intravenous conditioning" refers to the establishment of taste preferences for tastes paired with the intravenous delivery of nutrient. Fewer attempts have been made to establish taste preferences with this site of administration.

Of the three studies that have attempted to produce a taste preference with systemic infusions of glucose (Mather, Nicolaidis & Booth, 1978; Revusky, Smith & Chalmers, 1971; Tordoff & Friedman, 1986), only one study established a preference for the flavour paired with a glucose infusion (Mather, Nicolaidis & Booth, 1978). In the study by Mather et al (1978), rats received one flavour of chow paired with a systemic infusion of 30% glucose (weight/volume, w/v) and another flavour of chow paired with a systemic infusion of 0.9% saline (w/v) (Mather et al, 1978). Infusions began five minutes following the onset of eating and continued for an unspecified period of time. This protocol established a 73% preference for the flavour of chow that had been paired with glucose infusions (Mather et al, 1978).

A single study has attempted to establish taste preferences with glucose infusions into the portal vein, a vein that brings absorbed nutrient directly from the intestines to the liver (Tordoff & Friedman, 1986). This study was successful. Two hour infusions of glucose or saline paired with access to one of two distinct flavours of chow produced a four hour preference for the flavour of chow that had been paired with portal infusions of glucose. Other rats that received identical conditioning but had glucose and saline infused into the systemic circulation did not develop a flavour preference (Tordoff & Friedman, 1986). Tordoff & Friedman (1986) concluded that taste preferences are supported by portal but not systemic infusions of glucose.

Learned Control of Meal Size

There are numerous demonstrations that tastes, that have been paired with the postingestive consequences of eating, can control meal size. For example, rats that had consumed foods with flavours that reliably predict caloric density, consumed larger meals when a food was flavoured with a "low" calorie flavour than when the same food was flavoured with a "high" calorie flavour (Booth, 1972).

Similar taste-specific, learned control of meal size occurs in humans. Preschool children that had previously

eaten distinctively flavoured high and low calorie snacks, consumed larger meals when the meal was preceded by a snack flavoured with a "low" calorie flavour than when the meal was preceded by an identical snack flavoured with a "high" calorie flavour (Birch & Deysher, 1985). And adults who had consumed high and low calorie preloads consistently paired with distinct flavours of an isocaloric dessert, consumed more "low" calorie flavoured dessert than "high" calorie flavoured dessert, when challenged with an isocaloric preload (Booth, Lee & McAleavey, 1976).

Booth suggested that such taste-specific, conditioned increases in meal size occur because of a conditioned increase in the rate of ingestion at the end of the meal (Booth, 1972). He demonstrated that rats ate larger meals when solutions contained flavours previously associated with low caloric solutions because "low" calorie flavours produced higher rates of consumption than "high" calorie flavours at the end of a meal (Booth, 1972). Hence, he described conditioned, taste-specific increases in meal size as examples of "conditioned desatiation".

However, others have shown that conditioned changes in meal size can also occur at the beginning of the meal. These demonstrations were made with sham feeding preparations.

Sham feeding preparations are ideally suited to assessing the ability of taste to control feeding behavior.

In sham feeding, rats experience the taste of a food but the postingestive consequences of eating are eliminated or minimized (Sclafani & Nissenbaum, 1985). Diet ingested by mouth either drains out a fistula in a rat's esophagus (Mook & Wagner, 1987, 1991) or stomach (Weingarten & Powley, 1980), or is actively siphoned from rats' stomachs as they eat (Davis & Campbell, 1973).

Meal size is known to increase with repeated sham feeding experience. Several laboratories have shown that rats consume more solution on their second and third exposure to it under sham feeding conditions than in their first sham feeding trial (Davis & Campbell, 1973; Mook, Culberson, Gelbart & McDonald, 1983; Weingarten & Kulikovsky, 1989). These increases in meal size with successive sham feeding experience reflect increases in intake in the first ten minutes of the meal (Davis & Campbell, 1973; Weingarten & Kulikovsky, 1989). Microanalysis of rats' feeding behavior indicates that changes in licking behavior underlie increases in intake early in the meal. In the first sham feeding trial, rats generate high rates of licking that undergo a rapid, exponential decline in the first ten minutes of the meal (Davis & Smith, 1990). With repeated sham feeding experience, a slower linear decline replaces the initial exponential decline and intake increases (Davis & Smith, 1990).

The classic interpretation of increases in meal size with repeated sham feeding experience is that sham feeding, which presents a taste with no or few of its postingestive consequences, extinguishes a learned association between the taste of a food and its postingestive consequences (Davis & Campbell, 1973). Multiple lines of evidence support this "learning" interpretation.

First, the initial suppression of sham feeding intake is taste specific. Rats with extensive experience sham feeding one solution, suppress their sham feeding intake when allowed to sham feed a different solution (Davis & Campbell, 1973). This suggests that rats' sham feeding behavior is controlled by the taste of the solution and rules out the possibility that meal size increases with sham feeding experience merely because rats acquire a high drinking rate. Taste-specific suppression of intake also eliminates the possibility that sham feeding is suppressed by the novelty of food draining out the fistula.

Second, sham feeding intake is suppressed only if rats have previously "real fed" the solution and, thus, had the opportunity to associate the taste of a solution with its postingestive consequences (but see Mook et al, 1983). Weingarten & Kulikovsky (1989) trained rats to sham feed two distinctively flavoured sucrose solutions and then allowed them to real feed one of the solutions. When rats were re-exposed, on separate trials, under sham feeding conditions

to the two flavours of sucrose, sham feeding intake was suppressed only for the solution that had been previously real fed (Weingarten & Kulikovsky, 1989). The fact that rats must real feed a solution to suppress their initial intake of this same solution under sham feeding conditions also eliminates the possibility that rats suppress initial sham feeding intake because of neophobia.

Finally, pre-exposing rats to the taste of a solution impairs their ability to form taste-to-postingestive consequence associations. Rats that were pre-exposed to the taste of a solution under sham feeding conditions and then allowed to real feed the same solution (ie form a taste-to-postingestive consequence association) did not demonstrate taste-to-postingestive consequence learning. Specifically, they did not suppress their subsequent sham feeding intake of the solution that they had real fed (Weingarten & Kulikovsky, 1989). Pre-exposing rats to the taste of a solution provides CS pre-exposure, a standard technique for impairing the formation of CS-US associations in classical conditioning. The fact that CS pre-exposure prevented the initial suppression of sham feeding strongly suggests that the initial suppression of sham feeding intake is a learned phenomenon.

The establishment of taste-specific suppression of sham feeding as a "learned" phenomenon means that taste-specific suppression can be used as a behavioral probe for

the presence of a taste-to-postingestive consequence association.

Identifying the Unconditioned Stimulus in Taste-to-Postingestive Consequence Learning

Although the capacity for taste-to-postingestive consequence learning has been demonstrated, it is unclear which postingestive event(s) become associated with the taste of food during eating. A plethora of events follow the ingestion of food. Theoretically, any one of these events, in isolation or combination, could become associated with the taste of a food during eating and produce taste-to-postingestive consequence learning. This section reviews the relevance of various postingestive consequences in taste-to-postingestive consequence learning.

A) Calories

Bolles and colleagues advocated that calories generated by food during eating establish taste-to-postingestive consequence learning. They demonstrated that rats preferred flavours paired with the greatest caloric density whether caloric density was manipulated by diluting one food with chalk (Bolles, Hayward & Crandall, 1981; Hayward, 1983) or by increasing the concentration of one of

the nutritive solutions (Mehiel & Bolles, 1984). Further support for the role of calories in taste-to-postingestive consequence learning is provided by studies that show that rats do not develop a preference when exposed to isocaloric solutions (Mehiel & Bolles, 1984; Tordoff, Tepper & Friedman, 1987). But other studies show that isocaloric solutions can produce taste preferences. Rats that were exposed to isocaloric solutions of fructose and glucose preferred the flavour paired with the glucose (Ackroff & Sclafani, 1991) or fructose solution (Tordoff et al, 1990). Isocaloric solutions also differ in their ability to produce taste preferences. For example, flavours paired with intragastric administration of polycose produced a 95% preference after four days of conditioning (23 hours/day, Elizalde & Sclafani, 1990) but isocaloric infusions of oil failed to support a preference with similar conditioning procedures (Lucas & Sclafani, 1989). Thus, while rats may develop preferences based on caloric density, a consequence that covaries with caloric density may, in fact, support taste-to-postingestive consequence learning.

B) Stomach - Chemostimulation

Deutsch and colleagues suggested that stimulation of nutrient receptors in the stomach is sufficient to establish taste preferences during eating (Deutsch & Wang, 1977). They demonstrated that intragastric infusion of nutrient,

isolated to the stomach by a pyloric cuff, produced a taste preference (Deutsch & Wang, 1977). However, they only isolated nutrient to the stomach during conditioning (Deutsch & Wang, 1977). At the end of each conditioning trial, the pyloric cuffs were deflated, permitting nutrient egress from the stomach to the duodenum. Baker & Booth (1989) argued that rats' preferences for flavours paired with intragastric infusions of nutrient may have been based on taste associations with consequences beyond the stomach. By similar argument, demonstrations that taste preferences can be established by intragastric administration of nutrient do not _necessarily_ imply that a gastric consequence supports taste-to-postingestive consequence learning since nutrient delivered to the stomach also subsequently stimulates sites beyond the stomach.

C)) Small Intestine - Chemostimulation, Cholecystokinin

Booth & Davis (1973) suggested that rapid delivery of nutrient from the stomach to the duodenum early in a meal is responsible for taste-to-postingestive consequence learning. They demonstrated that rats exposed to meals that varied in caloric density in the first 5 minutes of the meal, learned to use the flavours added to these "high" and "low" calorie meals to control meal size and food choice. Rats ate larger meals when a new solution was flavoured with the "low calorie" flavour then when the "high calorie"

flavour was added to the same solution (Booth & Davis, 1973). Rats also acquired a preference for the "high calorie" flavour (Booth & Davis, 1973). Increasing the delivery of nutrient to the duodenum also facilitated learning. Vagotomized animals with increased rates of gastric emptying (Snowdon & Epstein, 1970) acquired greater taste-specific control of meal size and did so in fewer trials than sham vagotomized animals (Booth & Davis, 1973). Although Booth & Davis (1973) demonstrated that increasing the density, or amount, of nutrient delivered to the duodenum early in a meal produced taste-to-postingestive consequence learning, delivery of nutrient to the duodenum was not necessarily the consequence responsible for their demonstrations of taste-to-postingestive consequence learning. Rats can form taste-to-postingestive consequence associations when the UCS follows the CS immediately, or after a significant delay (eg. Elizalde & Sclafani, 1988). Varying delivery of nutrient to the duodenum may have produced another consequence minutes to hours after the onset of the meal and this consequence, and not delivery of nutrient to the duodenum, may have produced taste-to-postingestive consequence learning.

More convincing evidence for the relevance of an intestinal consequence in taste-to-postingestive consequence learning is provided by studies that demonstrate that the addition of acarbose to oral polycose solutions blocks the

acquisition of preferences for flavours paired with oral polycose (Elizalde & Sclafani, 1988) or gastric infusion of polycose (Sclafani, 1990). Acarbose is a drug which blocks the digestion and absorption of polycose in the small intestine. Demonstrations that acarbose blocks taste-to-postingestive consequence learning suggest that a relevant postingestive consequence in this phenomenon originates at the active site of acarbose: namely, the small intestine. The ability of acarbose to block the establishment of a flavour preference also suggests that gastric distension and gastric chemostimulation do not support taste-to-postingestive consequence learning since these signals are unaffected by acarbose. Unfortunately, the exact consequence in the small intestine that is blocked by acarbose, and hence the exact consequence necessary for taste-to-postingestive consequence learning, remains unknown.

Others have suggested that the secretion of cholecystokinin (CCK) on nutrient arrival in the small intestine is sufficient to establish taste-to-postingestive consequence learning (Mehiel & Bolles, 1988). But early studies that paired flavours with the exogenous administration of CCK either failed to produce a taste preference (Ervin & Teeter, 1986; Holt, Antin, Gibbs, Young & Smith, 1974) or produced a taste aversion (Deupree & Hsiao, 1987; Deutsch & Hardy, 1977). Recently, flavours

paired with low doses of CCK have established a 70% taste preference (Perez & Sclafani, 1991). Perez & Sclafani (1991) argued that they were able to produce a taste preference with exogenous CCK while others had failed because their procedures more closely approximated the conditions of endogenous CCK release during eating [when taste-to-postingestive associations are formed]. Rats used in earlier studies were food and water deprived and received CCK following the CS+ (Deupree & Hsiao, 1987; Deutsch & Hardy, 1977; Ervin & Teeter, 1986; Holt, Antin, Gibbs, Young & Smith, 1974). Rats used by Perez and Sclafani (1991) were solely deprived of food and received CCK during the ingestion of the CS+. Perez and Sclafani (1991) argued that rats enter meals hungry but not thirsty and that the release of CCK is triggered by nutrient arriving during, not following, a meal. While studies that establish a preference with exogenous CCK administration suggest that CCK may be a relevant consequence in taste-to-postingestive consequence learning, it remains to be established that doses of exogenous CCK that produce a taste preference mimic levels of endogenous CCK during eating.

D) Pancreas - Insulin

Insulin elevations were established as a putative consequence in taste-to-postingestive consequence learning with the demonstration that tastes paired, under sham

feeding conditions, with intraperitoneal (ip) injections of insulin, were strongly preferred to tastes that were sham fed and paired with ip injections of vehicle (Oetting & Vanderweele, 1985). Preferences established by insulin injections during sham feeding also reversed when the reinforcement contingencies were reversed (Oetting & Vanderweele, 1985). That is, when the former CS- flavour was paired with ip injection of insulin and the former CS+ flavour was paired with ip injection of vehicle, rats developed a preference for the former CS- flavour. But it is unclear whether insulin elevations support taste-to-postingestive consequence learning during real feeding since similar ip doses of insulin produced a taste aversion when paired with distinct flavours during real feeding (Vanderweele & Oetting Deems, 1989). Also, even if insulin elevations are a sufficient consequence to support taste-to-postingestive consequence learning, they are not a necessary consequence. Others have shown that rats preferred flavours of food paired with fructose relative to flavours paired with glucose (Tordoff, Ulrich & Sandler, 1990 but see Ackroff & Sclafani, 1991), despite the fact that fructose is the less insulinogenic sugar. And, diabetic rats, which lack insulin, still developed a preference for the flavour of chow paired with an oral fat emulsion (Tordoff, Tepper & Friedman, 1987).

E) Liver - Hepatic Fuel Oxidation

Macronutrients (except fat) are absorbed in the intestines and transported to the liver by the portal vein. The liver was identified as a relevant site for a consequence in taste-to-postingestive consequence learning when it was demonstrated that infusing glucose into the portal vein established a flavour preference. Tordoff & Friedman (1986) showed that rats preferred the flavour of food that had been paired with a portal infusion of glucose to the flavour that paired with a portal infusion of saline. Subsequent experiments by Tordoff and colleagues suggested that the specific consequence in the liver that established taste-to-postingestive consequence learning was hepatic fuel oxidation. First, they showed that diabetic rats, which can freely oxidize fat but have difficulty oxidizing glucose, developed an aversion to the flavour of chow that had been paired with oral glucose solutions but developed a preference for the flavour of chow that had been paired with oral fat emulsions (Tordoff, Tepper & Friedman, 1987). Second, they showed that rats preferred the flavour of chow that had been paired with fructose to the flavour of chow that had been paired with glucose (Tordoff et al, 1990). Rats' preference for flavours paired with fructose supports a hepatic fuel oxidation hypothesis, since fructose has been viewed to be preferentially metabolized by the liver (Niewoehner, Gilboe, Nuttal & Nuttal, 1984) and thus,

fructose would provide more fuel for oxidation than an isocaloric solution of glucose.

Although the data implicating the liver in taste preferences are impressive, the suggestion that increased hepatic fuel oxidation is a relevant consequence in taste-to-postingestive consequence learning is limited by the absence of critical experiments which directly measure, or manipulate, hepatic fuel oxidation during the acquisition of a taste preference.

F) Plasma Glucose Elevations

Eating produces elevations of plasma glucose (Smadja, Morin, Ferré & Girard, 1988; Strubbe & Steffens, 1977). Several studies have addressed whether rises in plasma glucose are sufficient to produce taste-to-postingestive consequence learning. With one exception (Mather et al, 1978), preferences were not established for flavours paired with systemic infusions of glucose (Revusky et al, 1971; Tordoff & Friedman, 1986). However, these negative results do not <u>necessarily</u> imply that postprandial elevations of plasma glucose elevations do not support taste-to-postingestive consequence learning. No study to date has documented that their plasma glucose manipulation mimics the time course of plasma glucose following a meal.

Thesis Focus and Outline of Experimentation

This thesis assesses the relevance of two putative consequences in taste-to-postingestive consequence learning.

First, I assess whether systemic infusions of glucose, calibrated to mimic the postprandial profile of plasma glucose, support taste-to-postingestive consequence learning. In Chapter 3, I allow rats to sham feed tastes that are paired with intravenous (iv) infusions of glucose that mimic real feeding. Then, taste-to-postingestive consequence learning is probed by testing for a suppression of sham feeding for tastes paired with iv infusions of glucose and/or the establishment of a preference for tastes paired with iv infusions of glucose. In the course of this investigation, I note that sham feeding itself produces rises in plasma glucose. In Chapter 4, I investigate the source of plasma glucose rises during sham feeding.

Second, I assess whether portal elevations of glucose, in isolation, are sufficient to establish taste-to-postingestive consequence learning. While others have demonstrated that rats prefer flavours of chow that have been paired with portal infusions of glucose relative to those paired with portal infusions of saline (Tordoff & Friedman, 1986) their protocol cannot rule out the possibility that one or more of the nutritive consequences of chow contributed to the development of a taste

preference. In Chapter 5, I assess the ability of portal infusions of glucose, in isolation, to support a taste preference by pairing flavour cues in non-nutritive solutions with portal infusions of glucose and saline.

CHAPTER II. GENERAL METHODS & MATERIALS

Subjects

Subjects in Experiments 1 to 5 were male, Long-Evans rats purchased from Charles River Inc. (Canada) or bred in the McMaster Psychology vivarium from animals purchased from Charles River. Subjects in Experiments 6 and 7 were male, Sprague-Dawley rats purchased from Charles River Inc (Wilmington, MA).

Rats were individually housed in hanging metal cages (Experiments 1 to 5) or transparent Plexiglas cages (Experiments 6 & 7). Colony rooms were maintained at 21°C on a 12:12 hour light/dark cycle. In Experiments 6 and 7, the colony room was illuminated with dim red lighting during the dark cycle to allow experiments to be conducted. Water was available ad lib in the animals' home cages. Food was provided in the home cages according to the experimental protocol (Purina Laboratory Rodent Chow pellets in Experiments 1 to 5; Purina Laboratory Rat Chow powder in Experiments 6 & 7).

Surgery

a. Gastric Cannulation

To permit sham feeding, rats were implanted with chronic, indwelling gastric cannulae. Each gastric cannula consisted of an 11 mm stainless steel tube (8.5 mm outer diameter [OD], 7.9 mm inner diameter [ID], flanged at both ends). Marlex mesh (2.5 cm x 2.5 cm), secured to the middle of the cannula shaft with dental cement 24 hours prior to surgery, which stabilized the cannula once in situ. A set screw threaded into the cannular shaft allowed the gastric cannula to be occluded and maintained the continuity of the gastrointestinal tract for real feeding.

Rats were deprived of food for 24 hours prior to gastric cannulation. Sodium pentobarbitol (Somnotol, loading dose: 65 mg/kg, intraperitoneal injection [ip]) was used to anaesthetize the animals. Atropine sulfate (0.2 ml of 0.6% solution, ip) was given at the onset of surgery to reduce salivary and mucous secretions.

To implant a gastric cannula, a 2.5 cm incision was made in the abdominal wall in mid line and the stomach exposed. Two concentric purse-string silk (5-0) sutures were sewn in the wall of the forestomach. A stab incision, in the centre of the purse-string sutures, permitted one end of the cannula to be inserted into the stomach and secured. The remaining free end of the cannula was brought through a

1 cm stab wound in the left abdominal wall and Marlex mesh (2.5 cm x 2.5 cm) applied over the free end of the cannula on the abdominal wall. The cannula was exteriorized with a 1 cm stab wound in the skin. A single silk (3-0) purse string surrounding the stab wound in the skin secured the exteriorized cannula. The cannula was closed with a set screw. The abdominal wall was closed with interrupted catgut (3-0) sutures and stainless steel wound clips were used to close the skin. Immediately following surgery, wounds were treated with a topical antibacterial gel (Furacin, Austin Laboratories Ltd., Canada). Animals were allowed at least 14 days to recover from surgery and were maintained on ad lib food during this period.

b. Jugular and Femoral Vein Catheterization

Jugular and femoral catheters were manufactured 24 hours prior to surgery. The distal end of the catheter, destined to be inserted into the vein, was 80 mm (jugular catheter) or 170 mm (femoral catheter) in length and composed of silicone (Silastic) tubing (0.3 mm ID, 0.6 mm OD). The proximal end of the "vein" tubing was inserted into a 3 mm length of silicone (Silastic) tubing (0.5 mm ID, 0.9 mm OD) before being inserted into another 10 mm length of silicone (Silastic) tubing (0.75 mm ID, 1.65 mm OD). The barrel of a 2.5 cm 20 gauge needle, bent to 90 degrees at midshaft and inserted into the proximal end of the catheter,

completed the catheter assembly. Silicone adhesive (Silastic type A) was used to seal all tubing junctions and to form a silicone ball on the outer wall of the catheter. This silicone ball prevented the catheter from advancing further than 3.2 cm in the jugular vein and 3.0 cm in the femoral vein. Catheters were filled with heparinized (50 U/ml) saline (0.15M) prior to surgery and were flushed daily, once in situ, with 0.1 ml of heparinized (50 U/ml) saline (0.15M).

To implant a catheter, rats were anaesthetized with sodium pentobarbitol (Somnotol, loading dose 65 mg/kg, ip). Atropine sulfate (0.2 ml of 0.6% solution, ip) was given at the onset of surgery to reduce salivary and mucous secretions. An antibiotic (0.3 ml Derapen-C, Ayerst Laboratories, Canada) injected intramuscularly during surgery in Experiments 2, 3 and 5, and on a weekly post-operative basis in Experiment 2, minimized bacterial infections.

Chronic jugular catheters were implanted using a procedure modified from Steffens (1969). In our procedure, a midline incision was made in the scalp, the skull exposed and four stainless steel screws inserted into the skull to anchor a headcap. The right jugular vein was exposed with a 2 cm incision, superior to the right clavicle. A catheter was passed, via a subcutaneous tunnel, from the right side of the scalp incision to the jugular dissection and

inserted, caudally, into the jugular vein until the tip rested in the superior vena cava at the right auricle. Two silk (5-0) sutures were used to secure the catheter to the vein at the point of insertion and quick drying adhesive (Krazy Glue) was used to seal the jugular vein incision. Interrupted catgut sutures were used to close the fascia over the jugular vein and the skin was closed with stainless steel wound clips. A headcap made from dental acrylic cemented the catheter to the skull. A 1 cm length of silicone (Silastic) tubing (0.75 mm ID, 1.65 mm OD), knotted at its end, covered the exposed end of the catheter. Animals were allowed at least three days to recover and were maintained on ad lib food during this period. Experiments began when animals demonstrated stable body weights.

Chronic femoral catheters were implanted with jugular catheters in Experiment 2. After preparing the skull for a headcap and dissecting the right jugular vein, the right femoral vein was exposed with a 2 cm incision that extended rostrally to the midpoint of the right inguinal ligament. The femoral catheter was passed with the jugular catheter from the scalp incision to the jugular dissection and advanced, caudally, to the femoral dissection via a midline subcutaneous tunnel. The jugular catheter was inserted, secured, and the wound closed as previously described. A small incision was made in the right femoral vein and the femoral catheter inserted rostrally until its

tip rested in the inferior vena cava. The femoral catheter was secured and the wound closed as per the jugular procedure. Animals were allowed at least three days to recover from surgery and were maintained on ad lib food during this period.

c) Portal vein catheterization

Portal catheters were manufactured from silicone (Silastic) and plastic (Tygon) tubing 24 hours prior to surgery. The distal end of the catheter, destined to be inserted into the vein, was 28 cm in length and composed of silicone (Silastic) tubing (0.5 mm ID, 0.9 mm OD). The proximal 7 cm of silastic tubing was coated with adhesive (Krazy glue) prior to being inserted into, and 3mm beyond, a 7 cm length of tygon tubing (1.0 mm ID, 1.8 mm OD). A piece of Marlex mesh (2.5 cm x 2.5 cm) secured to the midpoint of the tygon tubing with a single silk (3-0) suture, reinforced with adhesive (Krazy glue), stabilized the catheter once in situ. The shaft of a 23 gauge needle, inserted into the exposed, proximal end of the silastic tubing and secured with adhesive (Krazy glue), completed the catheter assembly. A 3 cm piece of tygon tubing (0.5 mm ID, 1.5 mm OD), occluded at its distal end with the metal hub of a 23 gauge monoject needle (Sherwood Medical, St. Louis, MO), served as a removeable cap for the catheter. Catheters were filled with 0.15M saline prior to surgery. Portal catheters were

flushed 24 hours following implantation with 0.01 grams of kanamyacin sulfate (Lyphomed, IL) suspended in 1 ml of 0.15M saline. Thereafter, catheter patency was maintained by flushing catheters daily with 0.2 ml of 0.15M saline.

To implant a portal catheter, rats were anaesthetized with a subcutaneous injection of a mixture of ketamine (Vetalar, loading dose 90 mg/kg) and acepromzine (Promace, 1 mg/kg). A low, 4 cm abdominal incision in midline was used to expose the ileocolic vein in the mesentery of the cecum. A portal catheter was then passed, via a subcutaneous tunnel, from a 1 cm incision at the ventral base of the neck to the ileocolic dissection. Silk (4-0) sutures were used to apply three point tension to the ileocolic vein above and below the origin of a collateral branch, and on the collateral branch itself. A small incision, made in the main trunk of the ileocolic vein at the origin of the collateral, allowed the catheter to be inserted into the ileocolic vein and advanced, rostrally, until its tip rested in the portal vein close to the liver. Two silk (4-0) sutures secured the catheter to the vein at the point of insertion and quick drying adhesive (Krazy Glue) sealed the vein incision. Continuous silk (3-0) sutures were used to close the abdominal wall and the skin was closed with stainless steel wound clips. The neck incision was closed with a single wound clip placed superior to the exiting catheter. Animals were allowed at least

seven days to recover from surgery and were maintained on ad lib food during this period. Experiments began when animals demonstrated stable body weights. The position of each portal catheter was verified at autopsy.

Sham and Real Feeding (Experiments 1 to 5)

Rats were deprived of food three hours before testing. To ensure a uniform level of deprivation, rats were trained to ingest 5 ml of liquid diet (Carnation Evaporated Milk diluted 1:1 with tap water) at the start of the deprivation period. All testing was conducted during the light phase of the light-dark cycle, between 12:30 and 17:00 hours.

Before testing, rats' stomachs were rinsed through the open cannulae with tap water (28°C). When rats were tested under sham feeding conditions, a 15 cm long stainless steel tube, lined with plastic tubing, was threaded into each cannula. This tube hung freely in the test cage and allowed ingested liquid food to drain out of the stomach. When rats were tested under real feeding conditions, the procedure was similar except that, after stomach lavage, the cannulae were closed so that food remained in the rats' stomachs.

Feeding tests lasted 30 minutes; intakes were recorded every five minutes. At the end of each feeding test in Experiments 1 to 4, animals were removed from the

test cages and returned to their home cages with the cannulae closed. Food was provided in their home cages immediately (Experiments 1 & 2) or within one hour (Experiment 3). In Experiment 5, once testing was complete, animals were sacrificed with a lethal ip dose of sodium pentobarbital (Somnotol, 65 mg/rat).

Rats sham, or real, fed 36% sucrose solutions (weight/volume). Sucrose solutions were distinctively flavoured, when required, with the addition of either 1.2 ml of almond or lemon extract (Club House Brand) per litre of solution. In Experiment 5, sucrose solutions were labelled with radioactive sucrose (0.33 μCi/ml, sucrose-UL-^{14}C, specific activity: 673 mCi/mmol, Sigma Chemical, St. Louis, MO).

Real feeding (Experiments 6A, 6B & 7)

Rats were deprived of food three hours before testing in Experiments 6A and 6B. In Experiment 7 rats were allowed ad lib access to food before testing. Testing began three hours into the dark phase of the light-dark cyle (Experiment 6) or at the onset of the dark cycle (Experiment 7). Animals were tested in their individual Plexiglas home cages (25 cm wide x 27 cm long x 30 cm high). Feeding tests lasted 30 minutes; intakes were recorded every five minutes. Rats real fed 0.15% (weight/volume) saccharin solutions (Experiments 6A, 6B, 7) or 18% (weight/volume) glucose

solutions (Experiment 7). Saccharin or glucose solutions were distinctively flavoured, when required, with the addition of either 1.2 ml of almond or lemon extract(Club House Brand) per litre of solution.

Blood Sampling and Infusions (Experiments 1, 2 & 5)

Blood sampling was conducted in a square (57 cm), ventilated, sound attenuating chamber that contained a Plexiglas cage (32 cm long x 10 cm wide x 31 cm high) suspended on 20 cm high stilts. A circular hole, on the front of the attenuating chamber and the Plexiglas cage, allowed the drinking spout of a graduated cylinder to be inserted 2.5 cm into the sham feeding cage. In order to collect blood without disturbing the animal, the rat's jugular catheter was attached, via silicone (Silastic) tubing, to a swivel (Small Animal Cannula Swivel 375, Instech Laboratories) suspended overhead by a counterbalanced pendulum. Other tubing, mounted on the same pendulum and connected to the femoral catheter, allowed fluid to be infused into the femoral vein in Experiment 2.

During blood sampling, the collecting tubing was filled with heparinized (50 U/ml) saline (0.15M). Immediately prior to drawing a 0.03 ml (Experiment 1) or a 0.5 ml sample of blood (Experiment 5), a volume of heparinized saline equal to the tubing dead space was removed. Heparinized (50 U/ml) saline (0.15M) equal to the

volume of the tubing dead space plus 0.03 ml (Experiment 1) or 0.25 ml (Experiment 5), was used to clear the tubing after each blood sample. This procedure kept the jugular catheter patent and allowed blood to be sampled over a two hour period. To obtain an accurate baseline measure of plasma glucose, blood was not sampled until each animal had been in the chamber, undisturbed, for an hour. Blood samples were then timed relative to the introduction of the drinking spout into the chamber and onset of feeding (Experiments 1,5) or the start of the test infusion (Experiment 2). Both of these events were designated as time 0. Blood was sampled at -10, 0, 5, 10, 15, 20, 30, 40 and 60 minutes during sham feeding in Experiment 1, and at four additional, consecutive, 15 minute intervals during real feeding in Experiment 1 and Experiment 2. Blood was sampled at -10, 0, 10, 20, 40, 60, 100 minutes in Experiment 5. Each blood sample was immediately centrifuged for 1 minute (10,750 rpm) and placed on ice. Blood sampling trials in experiments 1 and 2 were separated by at least four days.

Plasma Glucose and ^{14}C Assay

Plasma glucose concentration was determined with a Beckman II glucose analyzer using a glucose oxidase method. For ^{14}C determination, 100 μl samples of plasma were added to 4 ml counting fluid (ACS, Amersham, NY) and ^{14}C content

measured using a Beckman liquid scintillation counter (Model LS 5801) at a counting efficiency of 90%.

Infusions during Sham Feeding (Experiment 3)

Infusions were delivered to rats in individual, rectangular cages (20.5 cm long x 10 cm wide x 10 cm high) suspended on 19 cm high stilts. The floor of these cages was constructed of stainless steel rods which allowed sham feeding drainage tubes to pass through the floor. Test solutions were presented in graduated cylinders mounted on the outside wall of each test cage. The drinking spout of each graduated cylinder extended 2.5 cm into the test cage through a circular hole in the front wall of the cage. Test infusions were delivered via silicone (Silastic) tubing, suspended overhead, and connected to a swivel modelled after the swivel described by Brown, Amit & Weeks (1976).

Infusions during Real Feeding (Experiments 6 & 7)

Infusions were delivered to rats in their individual Plexiglas home cages (25 cm wide x 27 cm long x 30 cm high). Solutions were presented in graduated cylinders mounted on the outside wall of each cage. The drinking spout of each graduated cylinder extended 2.5 cm into the test cage through a circular hole in the front wall of the cage. Test infusions were delivered via plastic (Tygon) tubing, suspended overhead by a counterbalanced pendulum.

Assessment of Taste Preferences (Experiments 6 & 7)

Rats' preferences for almond and lemon-flavoured 0.15% saccharin solutions (Experiments 6 & 7) or almond and lemon-flavoured 18% glucose solutions (Experiment 7) were assessed in their home cages with a two bottle preference test. Testing was conducted at the same time of day and under the same food deprivation conditions as other trials in the given experiment. Two circular holes approximately 5 cm apart on the front of each animal's cage allowed the spouts of two graduated cylinders to be introduced into each animal's cage. One graduated cylinder contained the almond-flavoured solution; the other contained the lemon-flavoured solution. Rats' baseline taste preferences were assessed by recording intakes of the two solutions for 30 minutes. Rats' preferences were reassessed, according to the experimental protocol, by recording intakes of the two solutions for 30 minutes (Experiment 6) or 4 hours (Experiment 7). For the first 7.5 minutes of all preference tests, almond-flavoured solutions were presented on the left and lemon-flavoured solutions presented on the right. Thereafter, the positions of the solutions were reversed at 7.5 min intervals during the first 30 minutes of testing (Experiments 6 & 7) and subsequently at 30 minute intervals in hours one to four in Experiment 7. Reversing the positions of the tubes minimized the effect of animals' side preferences on intake. Intakes were recorded at 5, 15, and

30 minutes (Experiments 6 & 7) and at 30 minute intervals for hours 1 to 4 in Experiment 7.

CHAPTER III. DO PLASMA GLUCOSE ELEVATIONS SUPPORT TASTE-TO-POSTINGESTIVE CONSEQUENCE LEARNING?

In this series of experiments, I evaluate whether plasma glucose elevations are a sufficient consequence to support taste-to-postingestive consequence learning. Plasma glucose elevations were chosen for investigation because eating elevates plasma glucose (Smadja, Morin, Ferré & Girard, 1988; Strubbe & Steffans, 1977). Glucose is also an important metabolic fuel and one that is preferentially used and monitored by the brain (Mayer, 1955). Others have suggested, based on preference data, that elevations in plasma glucose do not support taste-to-postingestive consequence learning (Revusky, Smith & Chalmers, 1971; Tordoff & Friedman, 1986). Their tests are limited, however, because they fail to demonstrate that their manipulations of plasma glucose mimic the time course of plasma glucose following a meal.

EXPERIMENT 1

I begin by recording plasma glucose levels during sham and real feeding. Others have shown that real but not sham feeding produces taste-to-postingestive consequence

learning (Weingarten & Kulikovsky, 1989). Thus, real but not sham feeding must provide the rat with the relevant consequence for taste-to-postingestive consequence learning. Therefore, a preliminary test for the relevance of plasma glucose elevations in taste-to-postingestive consequence learning is to determine if plasma glucose elevations differ in sham and real feeding.

METHOD

Subjects were five rats weighing 230-300 grams at gastric cannula implantation and 380-490 grams at jugular vein catheterization.

Rats were trained to sham feed almond-flavoured 36% sucrose. When their 30 minute intakes had stabilized, rats were implanted with jugular catheters. Three or more days later they were individually adapted to the testing procedures. Training and blood sampling trials were identical except that blood was not sampled during training trials. On all trials, animals were prepared to sham feed and placed in the sound-attenuating chamber two hours after being food deprived. One hour later, the spout of a graduated cylinder containing almond-flavoured 36% sucrose was introduced into the chamber for 30 minutes. Training trials were given once a day, on successive days, until animals reliably initiated a meal as soon as the drinking

spout was introduced into the chamber; this was accomplished in two to three trials.

Rats were tested twice under sham feeding conditions and then tested once under real feeding conditions. Real feeding trials were identical to sham feeding trials except that the cannula was closed throughout the trial and blood was sampled for four additional 15 minute intervals, increasing the period of blood sampling from one to two hours.

RESULTS

Five animals completed sham feed testing. Two animals completed real feed testing.

Figure 1 shows cumulative intakes during sham and real feeding. As expected, when sham feeding, rats ate at a high rate for the entire 30 minute period of sucrose access. When real feeding, rats stopped eating within 15 minutes of meal initiation.

Plasma glucose values for each sampling trial were expressed as deviations from baseline values at 0 minutes. Plasma glucose deviations for individual sham feeding trials are presented Figure 2. Mean plasma glucose deviations for sham and real feeding are presented in Figure 3. Inspection of Figures 2 & 3 reveals that plasma glucose increased within five minutes of meal initiation during both sham and real feeding. In fact, sham and real feeding produced

Figure 1: Mean cumulative intake (+/- SEM) of 36% sucrose solutions during sham and real feeding (SEM's not visible fall within the symbol). SF(AVG)= average intake on 2 sham feeding trials, RF = intake on the first real feeding trial.

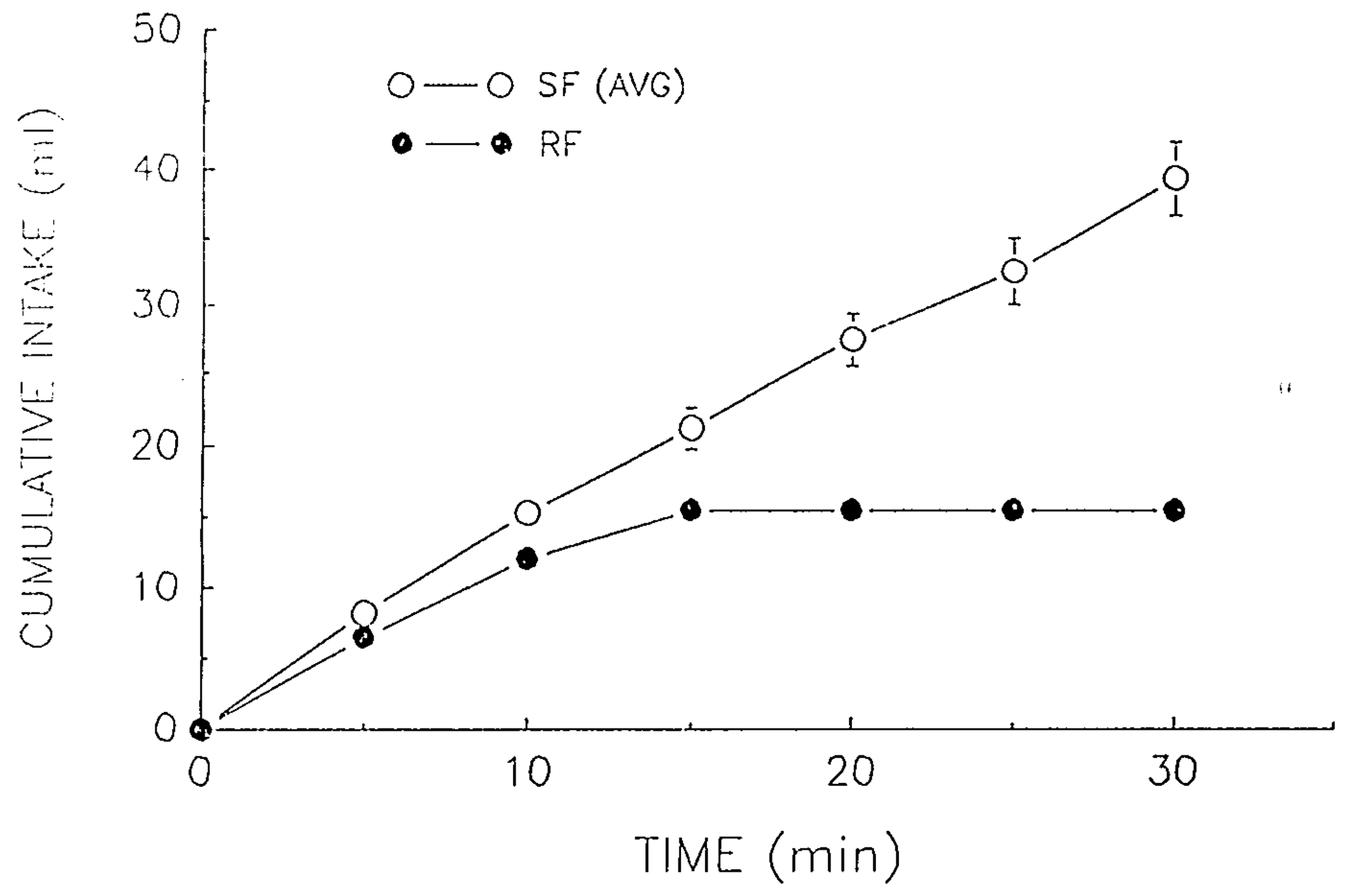
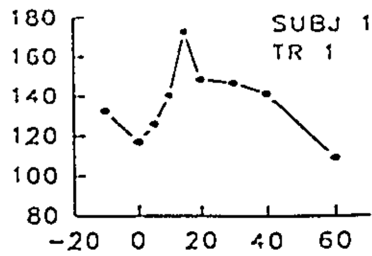
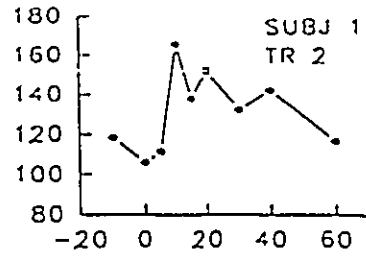
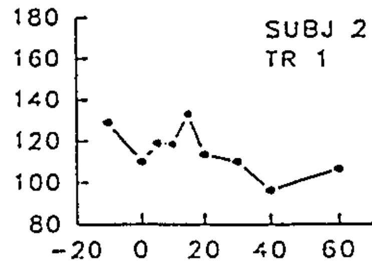

SF (AVG)
RF
CUMULATIVE INTAKE (ml)
TIME (min)
0
10
20
30
40
50

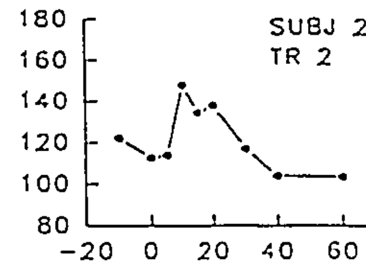
Figure 2: Deviations in plasma glucose, calculated from baseline values at 0 minutes, during individual sham feeding trials. Animals were given 30 minute access to 36% sucrose solutions at 0 minutes.

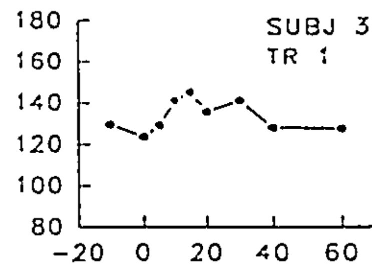
PLASMA GLUCOSE (mg/dl)

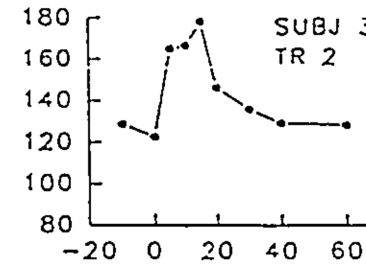


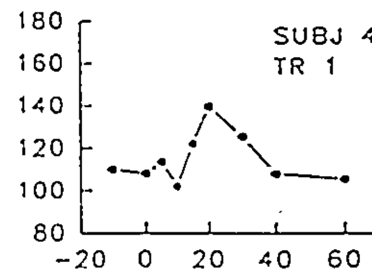


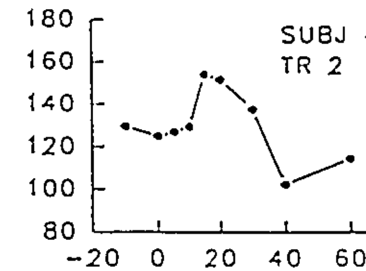


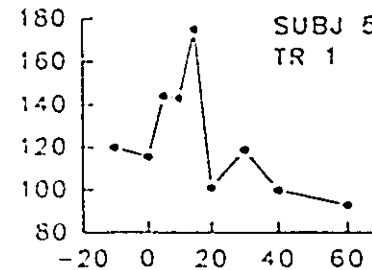






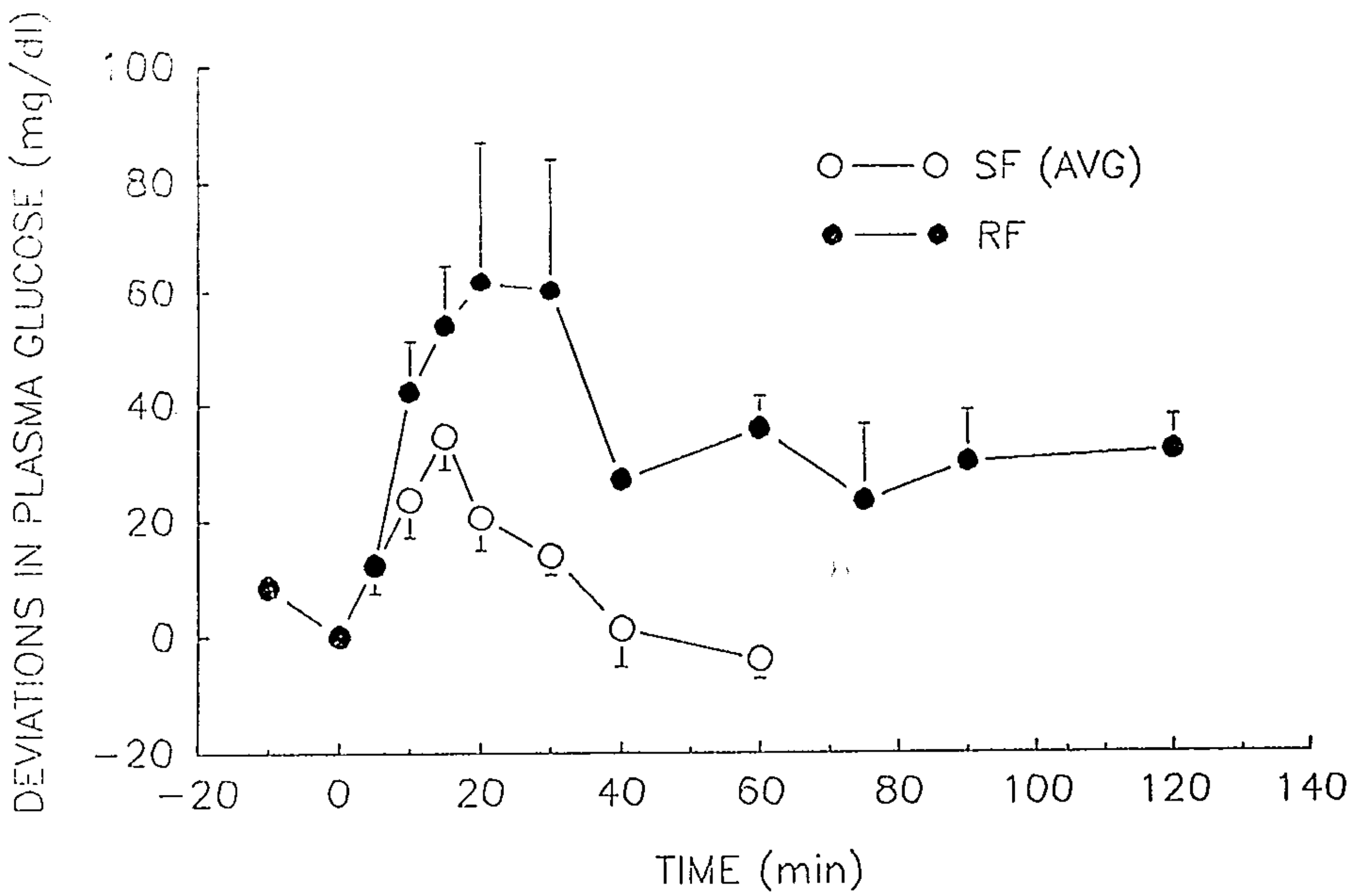






TIME (min)

Figure 3: Mean deviations in plasma glucose (+/- SEM), from baseline values at 0 minutes, during sham and real feeding. Average absolute baselines at 0 min: sham feeding (109.5 mg/dl), real feeding (115.6 mg/dl). SF(AVG) = average intake on 2 sham feeding trials, RF = intake on the first real feeding trial.



SF (AVG)
RF
DEVIATIONS IN PLASMA GLUCOSE (mg/dl)
100
80
60
40
20
0
−20
−20
0
20
40
60
80
100
120
140
TIME (min)

similar rises of plasma glucose from 0 to 15 minutes. Deviations in plasma glucose at 15 minutes were not significantly different when compared with a matched t test ($t(1) < 1$, NS). Subsequently, however, sham and real feeding produced different plasma glucose profiles. At 15 minutes, under sham feeding conditions plasma glucose began a precipitous decline. While plasma glucose also declined during real feeding, the plasma glucose value at 120 minutes during real feeding remained elevated relative to its baseline value at 0 minutes ($t(1)=-4.93$, $p<.01$).

DISCUSSION

This experiment demonstrates that sham feeding elevates plasma glucose, an effect that has been reported before (Berthoud, 1984; Sclafani & Nissenbaum, 1985). Although elevations in plasma glucose during sham feeding may reflect the absorption of exogenous nutrient, oral stimulation during sham feeding may also trigger a release of endogenous glucose. In Chapter 4, I investigate the source of plasma glucose rises during sham feeding.

The main purpose of this experiment was to compare levels of plasma glucose during real feeding which provides the necessary consequence for taste-to-postingestive consequence learning and sham feeding, a condition that fails to provide a relevant consequence for taste-to-postingestive consequence learning (Weingarten & Kulikovsky,

1989). The fact that both sham and real feeding produced markedly similar rises in plasma glucose in the first 15 minutes of eating suggests that rises in plasma glucose in this period are not a relevant consequence in taste-to-postingestive consequence learning. Relative to sham feeding, however, real feeding produced sustained elevations in plasma glucose for 120 minutes. Perhaps prolonged elevations in plasma glucose are a relevant postingestive consequence in taste-to-postingestive consequence learning. Subsequent experiments in this chapter investigate this possibility.

EXPERIMENT 2

Experiment 2 identifies the infusion rate required to produce the sustained elevations of plasma glucose characteristic of real feeding. This experiment is prerequisite to Experiment 3 which mimics the plasma glucose profile of real feeding in the sham feeding rat. Pilot work suggested that an infusion of 10% glucose at 0.037 ml/min would provide a plasma glucose profile similar to that observed during real feeding. This experiment confirms this possibility.

METHOD

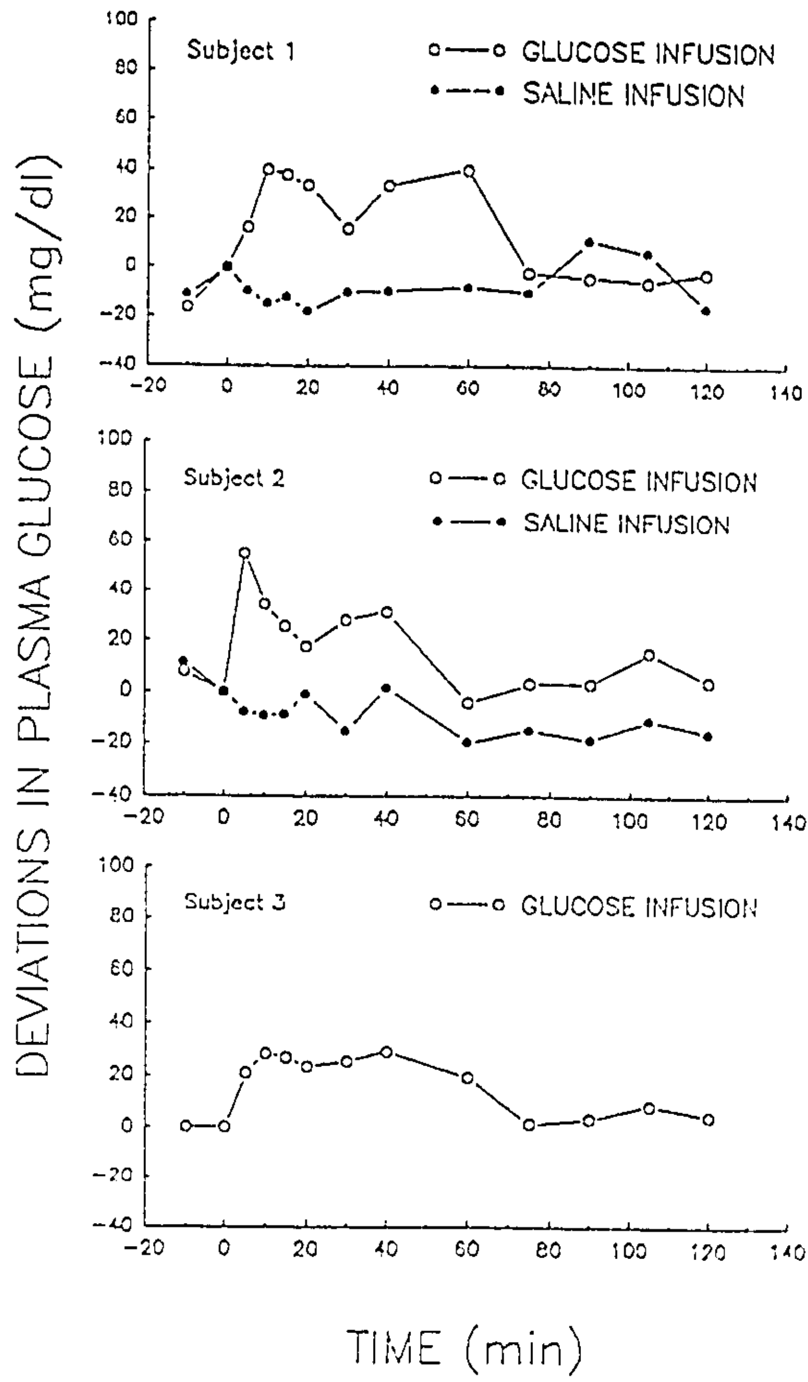
Subjects were three rats, weighing 295-335 grams at gastric cannula implantation and 440-505 grams at jugular and femoral catheterization. Jugular and femoral catheters were implanted at least fourteen days following the implantation of gastric cannulae.

Rats were adapted to the blood sampling cage for two days. Although animals did not sham feed in this experiment, their stomachs were rinsed as if they were going to sham feed in order to mimic the protocol of Experiment 1. Trials commenced after rats had been in the chamber undisturbed for one hour. On the first blood sampling trial, 10% glucose was infused into the femoral vein (0.037 ml/min) for 60 minutes while blood was sampled from the jugular vein. To test for the nonspecific effects of an infusion, 0.9% saline was subsequently infused into the femoral vein (0.037 ml/min) of two animals.

RESULTS & DISCUSSION

Figure 4 shows plasma glucose levels during glucose and saline infusions. Plasma glucose levels increased within five to ten minutes of the onset of an infusion of 10% glucose at 0.037 ml/min. Plasma glucose levels remained elevated for the duration of the infusion and returned to baseline 15 minutes after the infusion was terminated. Plasma glucose levels were not elevated due to a nonspecific

Figure 4: Deviations in plasma glucose, calculated from baseline values at 0 minutes, in individual animals during infusions of 10% glucose and 0.9% saline. Infusions (0.037 mls/min) began at 0 min and terminated at 60 min.


Subject 1
GLUCOSE INFUSION
SALINE INFUSION
Subject 2
GLUCOSE INFUSION
SALINE INFUSION
Subject 3
GLUCOSE INFUSION
DEVIATIONS IN PLASMA GLUCOSE (mg/dl)
TIME (min)
100
80
60
40
20
0
-20
-40
-20
0
20
40
60
80
100
120
140

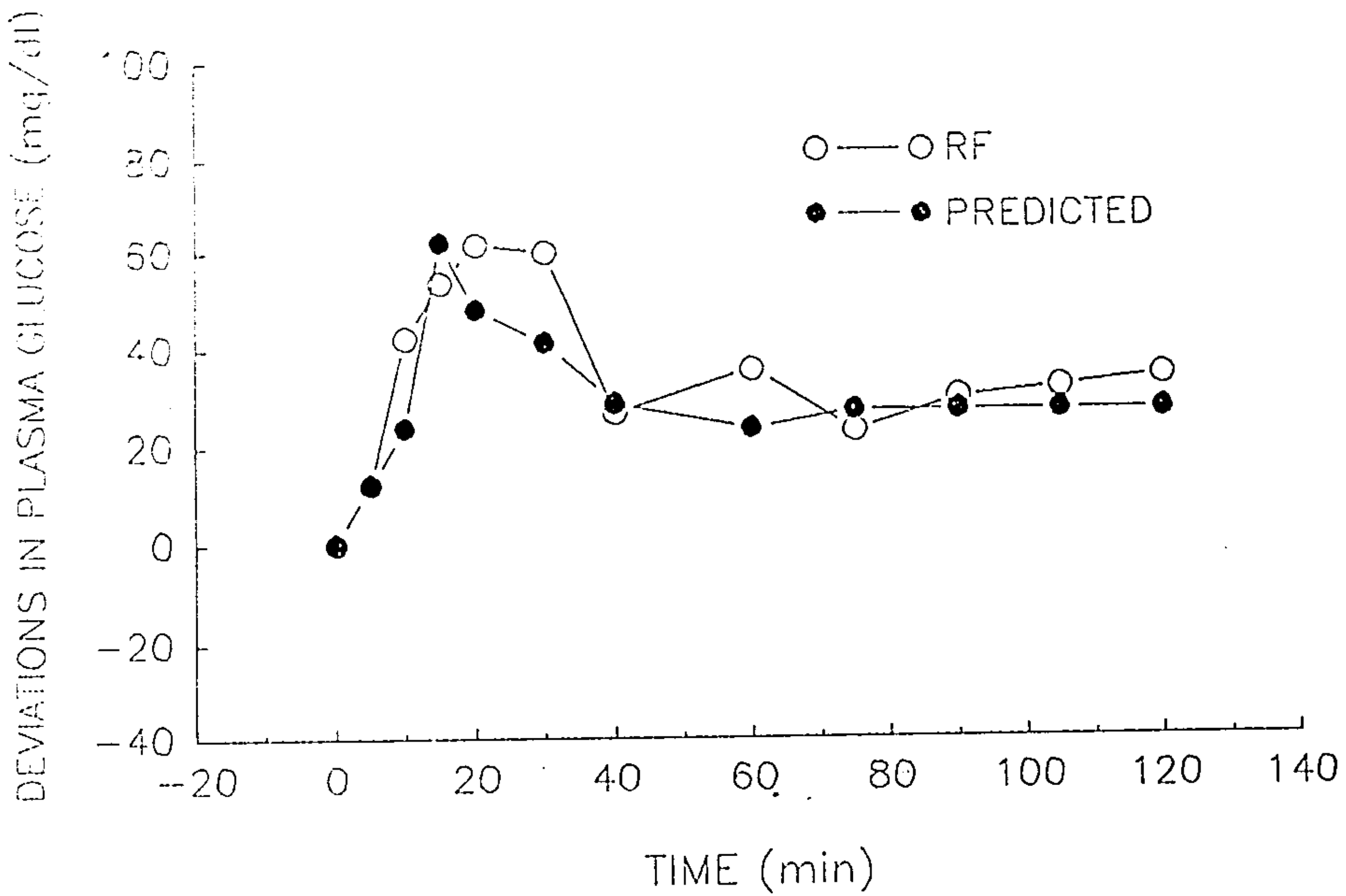
effect of an intravenous (iv) infusion since iv saline infusions did not elevate plasma glucose.

The aim of this study was to determine a glucose infusion rate that would mimic, in the sham feeding rat, the plasma glucose levels of real feeding. To validate that my infusion parameter accomplished this goal, I calculated the average deviation of plasma glucose from baseline, at minutes 5 through 60, during glucose infusions. This average deviation was added to the deviations of plasma glucose produced by 30 minutes of sham feeding alone (see Figure 3). Figure 5 shows the predicted plasma glucose profile produced by infusing 10% glucose at 0.037 ml/min in a sham feeding rat if the infusion begins 10 min after sham feeding is initiated. As Figure 5 indicates, this protocol produces a plasma glucose curve similar to that produced by real feeding.

EXPERIMENT 3

Experiment 3 assesses whether mimicking the plasma glucose profile of real feeding in the sham feeding rat produces taste-to-postingestive consequence learning. Others have shown that real but not sham feeding provides the rat with the necessary postingestive consequence(s) to form a taste-to-postingestive consequence association (Weingarten & Kulikovsky, 1989). If plasma glucose

Figure 5: Mean deviations in plasma glucose during real feeding (RF) and the predicted profile (Predicted) of plasma glucose values for sham feeding during the infusion of 10% glucose (0.037 msl/min). The predicted profile was computed by adding the average deviation in plasma glucose produced by a 10% glucose infusion (Experiment 2) to the mean deviations in plasma glucose produced by sham feeding (Experiment 1). These calculations were based on a 110 minute infusion of 10% glucose, beginning 10 minutes after access to a 36% sucrose solution.


RF
PREDICTED
DEVIATIONS IN PLASMA GLUCOSE (mg/dl)
80
60
40
20
0
−20
−40
−20
0
20
40
60
80
100
120
140
TIME (min)

elevations during real feeding establish taste-to-postingestive consequence learning then providing the sham feeding rat with the plasma glucose profile of real feeding should also produce taste-to-postingestive consequence learning. Plasma glucose levels were manipulated during sham feeding by infusing glucose intravenously at a rate shown, in Experiment 2, to mimic the plasma glucose profile of real feeding. The presence of a taste-to-postingestive consequence association was indexed with the two established probes for taste-to-postingestive consequence learning: namely, suppression of sham feeding intake for tastes previously paired with glucose infusions and the development of a preference for tastes previously paired with glucose infusions.

METHOD

Subjects were nine rats, weighing 265-305 grams at gastric cannulae implantation and 390-445 grams at jugular vein catheterization.

The experiment had two phases. In Phase 1, rats were trained to sham feed 36% unflavoured sucrose. When their 30 minute intakes had stabilized, rats were given a one-day exposure to lemon and almond-flavoured 36% sucrose. This exposure was provided to minimize neophobia on exposure to the flavoured sucrose solutions in Phase 2. Flavour exposure was counterbalanced so that half the animals sham

fed lemon sucrose on the first day and the others sham fed almond sucrose. They were exposed to the second flavour the following day in a counterbalanced fashion. Jugular catheters were implanted at the end of Phase 1.

The purpose of Phase 2 was to observe the effect of pairing prolonged elevations of plasma glucose with tastes under sham feeding conditions. Animals sham fed unflavoured 36% sucrose for four days. Then, two groups, matched for intake during these four days, were formed. For the next ten days, each group sham fed one flavour of 36% sucrose while receiving an intravenous infusion of 10% glucose (.037 ml/min) and sham fed the other flavour of 36% sucrose while receiving an intravenous infusion of 0.9% saline (.037 ml/min). Rats were named for the flavour of sucrose solution paired with the glucose infusion. Thus, LEMON rats sham fed lemon-flavoured 36% sucrose solutions while receiving a 10% glucose infusion; ALMOND rats sham fed almond-flavoured 36% sucrose while receiving a 10% glucose infusion. Saline and glucose infusions were presented in a ABBA sequence starting with a glucose infusion for both groups. Animals received one infusion/day. Infusions began 10 minutes after sucrose access and continued for 110 minutes. The day after the last infusion, animals' preference for almond and lemon-flavoured 36% sucrose was assessed, under sham feeding conditions, with a two-bottle

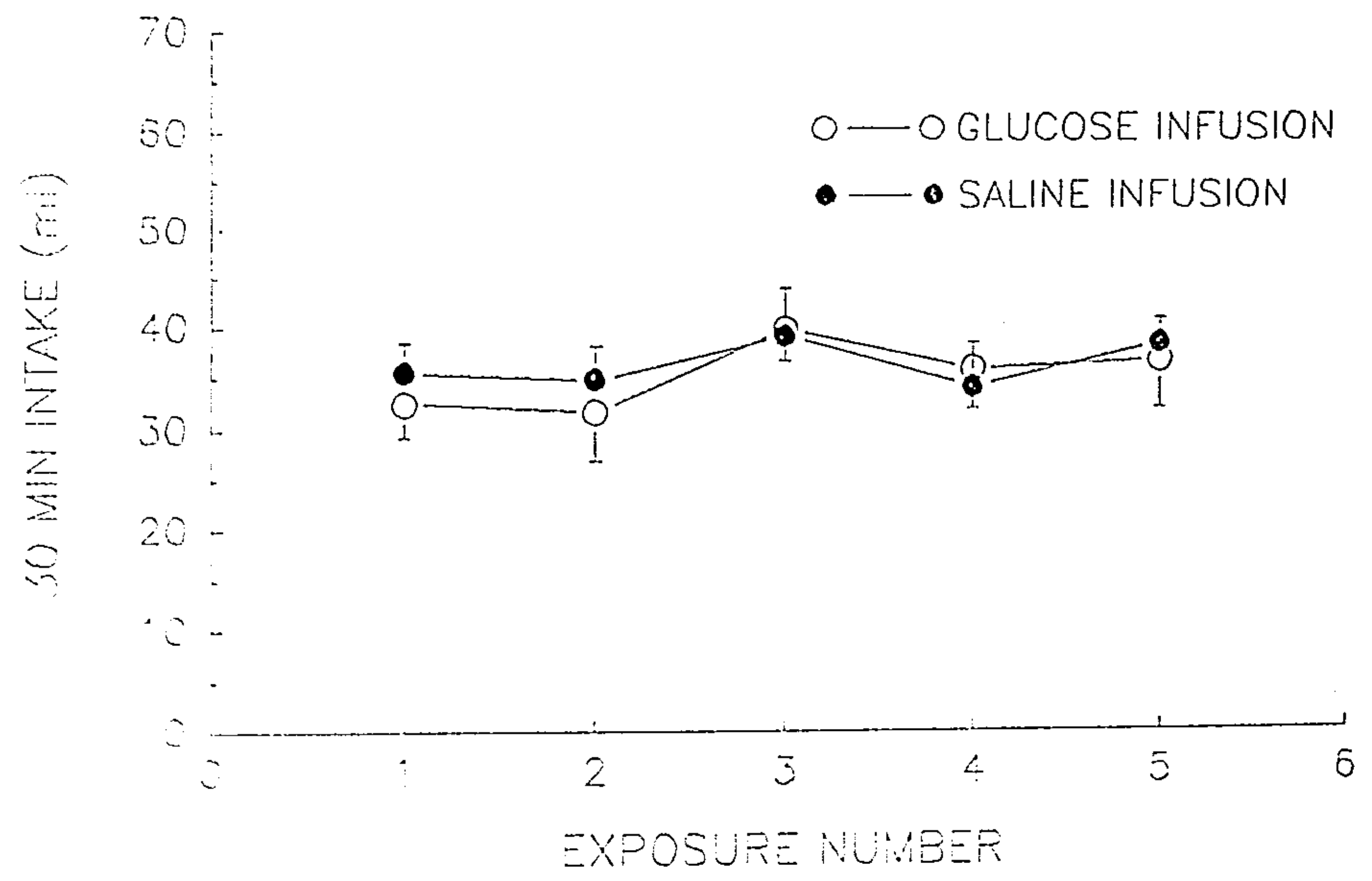
choice test. Side preference was controlled by alternating the position of flavours in successive cages.

RESULTS

Sucrose consumption during glucose and saline infusions was analyzed with a 3 way ANOVA with Flavour Group (almond vs lemon) as a between-subjects factor and Infusion (glucose vs saline) and Exposure Number (1 to 5) as within-subjects factors. ALMOND (N=4) and LEMON rats (N=5) consumed similar amount of sucrose during infusions (Flavour Group x Infusion, $F(1,7)=3.54$, NS) and across trials (Flavour Group x Exposure Number, $F(1,7)<1$, NS). Sucrose intake during glucose and saline infusions is presented in Figure 6. Sucrose intake did not decrease across trials (Infusion x Exposure Number, $F(1,7)<1$, NS).

Animals also did not develop a preference for the flavour that had been paired with glucose infusions. When preference was assessed with a two-bottle preference test, neither the amount consumed ($t(8)<1$, NS) nor the simple presence or absence of a preference (4 of the 9 rats preferred the flavour that had been paired with glucose infusions) indicated any bias for the flavour paired with glucose infusions.

Figure 6: Mean 30 minute intake of 36% sucrose solutions (+/- SEM) during infusions of 10% glucose and 0.9% saline. Infusions began 10 min after sucrose access and continued for 110 min.


GLUCOSE INFUSION
SALINE INFUSION
30 MIN INTAKE (ml)
70
60
50
40
30
20
10
0
1
2
3
4
5
6
EXPOSURE NUMBER

DISCUSSION

This experiment suggests that elevations of plasma glucose, in isolation, are not a sufficient consequence for taste-to-postingestive consequence learning. Infusions of 10% glucose, at a rate demonstrated to produce plasma glucose levels similar to those observed during real feeding, did not suppress sham feeding intake, a behavioral marker for taste-to-postingestive consequence learning. Since it was possible that the high palatability of 36% sucrose may have masked the expression of an association that would decrease intake, I also tested for a preference for the flavour that had been paired with glucose infusions. No preference was seen. These negative results either imply that rats in this study were incapable of learning to associate flavours with postingestive consequences or that plasma glucose elevations are not a sufficient postingestive consequence for taste-to-postingestive consequence learning. The former possibility is ruled out in Experiment 4.

EXPERIMENT 4

Experiment 4 tests whether animals used in Experiment 3 can form an association between a flavour and its postingestive consequence(s). In this experiment, the postingestive consequence(s) is "delivered" by allowing animals to real feed flavoured 36% sucrose. Taste-specific

suppression of sham feeding is used as the behavioral marker for taste-to-postingestive consequence learning (Weingarten & Kulikovsky, 1989).

METHOD

Nine rats who completed Experiment 3 were used. For five days, rats real fed, for 30 minutes, the flavour of 36% sucrose that had been paired with glucose infusions in phase 2 of Experiment 3. Thus, LEMON rats real fed lemon-flavoured 36% sucrose and ALMOND rats real fed almond-flavoured 36% sucrose. Both groups were then given two sham feeding trials with almond and lemon-flavoured 36% sucrose. Animals sham fed one solution/day and sham fed each flavour once. Flavour exposure was counterbalanced by alternating flavour across successive cages and presenting the opposite pattern the following day.

RESULTS

On the first real feeding trial, animals were hyperphagic compared to subsequent real feeding trials. Animals consumed a significantly larger meal (15 mls) on the first real feeding trial compared to the fifth real feeding trial (9.4 mls), ($t(8)=3.55$, $p<.01$).

When permitted to sham feed 36% sucrose, rats consumed significantly less of the flavour that they had

real fed (26.3 mls) compared to the flavour they had only sham fed (32.8 mls), ($t(7)=-3.8$, $p<.01$).

DISCUSSION

This experiment establishes that animals used in Experiment 3 were able to form a taste-to-postingestive consequence association if provided with a relevant consequence(s). Animals sham fed less of the flavoured solution that had been paired with a consequence(s) in real feeding than the flavoured solution that had been paired with no consequence(s) in sham feeding. This eliminates the possibility that animals in Experiment 3 failed to demonstrate taste-to-postingestive consequence learning because they were unable to form a taste-to-postingestive consequence association. One must conclude that elevations in plasma glucose are not a sufficient postingestive consequence to support taste-to-postingestive consequence learning.

This experiment also demonstrates that the experimentally-induced elevations in plasma glucose in Experiment 3 did not affect the real feeding behavior of animals in this experiment. Rats with a history of sham feeding are known to be hyperphagic on their first real feeding trial, (Van Vort & Smith, 1987; Weingarten & Kulikovsky, 1989). The fact that animals in this experiment were hyperphagic on their first real feeding trial implies

that the experimentally-induced elevations of plasma glucose, provided in Experiment 3, did not provide rats with a relevant satiety signal; animals behaved on the first real feeding trial as if they had only been sham feeding throughout Experiment 3.

CHAPTER IV. DO PLASMA GLUCOSE RISES DURING SHAM FEEDING REFLECT THE ABSORPTION OF EXOGENOUS GLUCOSE?

During sham feeding, in the gastric cannulated rat preparation, rats ingest a liquid diet by mouth that drains out a fistula in their stomachs. An assumption that underlies the use of this preparation in feeding research is that _all_ ingested diet drains out the gastric fistula and, therefore, no nutrient is absorbed. Two empirical findings support this assumption. First, when the dry weight of nutrient collected from the fistulae was compared to the dry weight of the ingested nutrient, drainage was 100.6 +/- 3.55% efficient (Kraly, Carty & Smith, 1978). Second, when dye was added to the ingested diet, 102.4 +/- 3.05% of the dye was recovered in the nutrient collected from the fistulae (Kraly et al, 1978).

Yet I, (Experiment 1) and others (Berthoud, 1984; Sclafani & Nissenbaum, 1985) have shown that plasma glucose rises in the gastric cannulated rat during sham feeding. These rises are also substantial; plasma glucose can rise by 30 to 50 mg/dl relative to baseline values during sham feeding (Experiment 1; Sclafani & Nissenbaum, 1985). Moreover, as I noted in Experiment 1, plasma glucose elevations in the first fifteen minutes of sham feeding are

not significantly different from those recorded in the first fifteen minutes of real feeding.

Why do plasma glucose levels rise in the gastric cannulated rat preparation during sham feeding? One possibility is that sham feeding allows nutrient to enter the duodenum where it is absorbed. Thus, plasma glucose rises during sham feeding reflect the absorption of exogenous glucose. Another possibility is that plasma glucose rises during sham feeding because endogenous glucose is released. A final possibility is that plasma glucose rises during sham feeding because exogenous glucose is absorbed and endogenous glucose is released.

Sclafani and Nissenbaum (1985) suggested that plasma glucose rises during sham feeding reflect the absorption of exogenous glucose. They demonstrated that plasma glucose levels do not rise during sham feeding when i) acarbose, a drug that prevents the digestion of sucrose, and hence glucose absorption, is added to the drinking solution (Sclafani & Nissenbaum, 1985) or ii) when nutrient is prevented from entering the duodenum by occluding the pylorus (Sclafani and Nissenbaum, 1985). Further support for their position is provided by studies that demonstrate that saccharin does not elevate plasma glucose when it is ingested (Berthoud, Bereiter, Trimble, Siegel & Jeanrenaud, 1981; Berthoud, Trimble, Siegel, Bereiter & Jeanrenaud,

1980, Tordoff & Friedman, 1989). Saccharin, a non-nutritive solution, cannot provide exogenous glucose for absorption.

However, plasma glucose rises during sham feeding may reflect an endogenous release of glucose. I demonstrated in Experiment 1 that plasma glucose elevations in the first fifteen minutes of sham and real feeding are similar. Food contact can also shift respiratory quotients (RQ) from values compatible with fat metabolism to values compatible with carbohydrate metabolism (Nicolaidis, 1969). Whether RQ shifts following food contact reflect the metabolism of endogenous or absorbed carbohydrate is an empirical question but the speed of the shift suggests that endogenous glucose is being metabolized since exogenous glucose would take time to be absorbed.

The release of endogenous glucose could be a cephalically mediated response. The sight, smell and taste of food is known to produce autonomic and endocrine responses that precede the absorption of food (for a review see Powley & Berthoud, 1985). While it has not been established that there is a cephalic response that liberates glucose, many other secretions such as insulin (Sjöström, Garellick, Krotkiewski & Luyckx, 1980), gastric acid (Farrel, 1928), and saliva (Pavlov, 1902) have a cephalic component to their response and the oropharyngeal stimulation associated with sham feeding may trigger a cephalically-mediated release of glucose.

A candidate for liberating endogenous glucose is liver glycogen. Hepatic glucose production has been shown to increase dramatically within three minutes of an intragastric infusion of glucose (Smadja et al, 1988). These increases in hepatic glucose production may reflect glycogenolysis since others have shown that liver glycogen content decreases ten (Langhans, 1991) or thirteen minutes following meal initiation (Langhans, Geary, & Scharrer, 1982).

In this experiment, I examine the source of plasma glucose elevations during sham feeding by testing for the absorption of exogenous glucose. I allow animals to sham feed a radiolabeled sucrose solution and monitor the radioactivity of their plasma. The predictions for this experiment are straightforward: If absorption of exogenous glucose contributes to plasma glucose rises during sham feeding, plasma radioactivity should increase during sham feeding. Conversely, if plasma glucose rises during sham feeding result solely from the release of endogenous glucose, plasma radioactivity should not increase during sham feeding.

METHOD

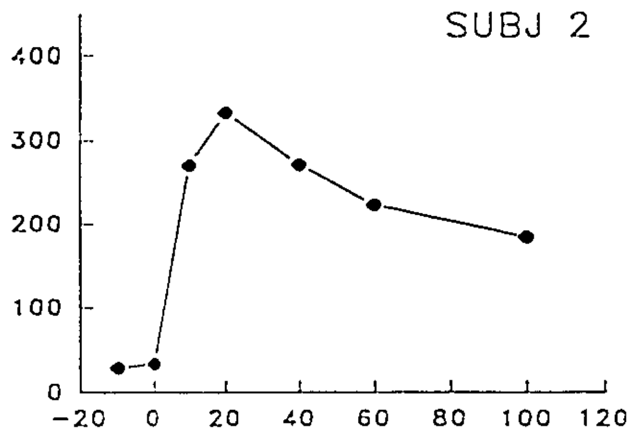
Subjects were three rats weighing 300 grams at gastric cannula implantation and 440-460 grams at jugular vein catheterization. Rats were trained to sham feed

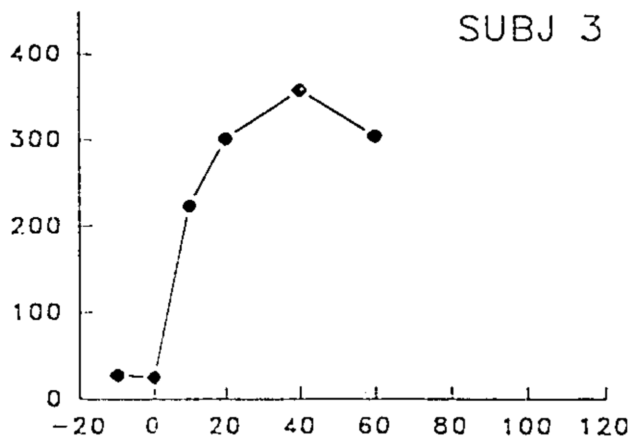
almond-flavoured 36% sucrose. Then they were implanted with jugular catheters and habituated to the testing procedures as in Experiment 1. Test conditions were identical in this experiment to those of Experiment 1 except that the drinking solution was labelled with a sucrose radioisotope (sucrose-UL-^{14}C) on blood sampling days.

RESULTS & DISCUSSION

The three subjects sham fed 37, 49 and 40 mls of 36% sucrose in the 30 minute session. These intakes correspond to the ingestion of 12.3, 16.3, and 13.3 μCi of ^{14}C-label. Figure 7 shows levels of plasma ^{14}C during sham feeding for each animal. Sham feeding produced elevations of plasma ^{14}C, relative to baseline values, within 10 min of feeding. Since sucrose is digested by the enzyme sucrase, found only in the small intestine (Leninger, 1982), elevated plasma ^{14}C indicates that, in the gastric fistulated rat, sucrose must reach the small intestine where it is digested and absorbed. Thus, this experiment provides additional evidence that plasma glucose rises during sham feeding (Experiment 1; Berthoud, 1984; Sclafani & Nissenbaum, 1985a) reflect the absorption of exogenous glucose. Whether plasma glucose rises during sham feeding <u>also</u> reflect the release of endogenous glucose cannot be determined from this experiment.

Figure 7: Plasma ^{14}C content during individual sham feeding trials. Animals had 30 minute access to 36% sucrose, labelled with sucrose-UL-^{14}C (0.33μCi/ml), beginning at 0 minutes.





PLASMA ^{14}C CONTENT (dpm)

TIME (min)

CHAPTER V. DO PORTAL ELEVATIONS OF GLUCOSE SUPPORT TASTE-TO-POSTINGESTIVE CONSEQUENCE LEARNING?

Taste-to-postingestive consequence learning does not occur when tastes are paired with elevations of plasma glucose (Chapter 3). While plasma glucose elevations may not support taste-to-postingestive learning, glucose elevations more proximate to the site of nutrient absorption may be sufficient to support a taste-to-postingestive consequence association. Glucose is absorbed in the small intestine and transported to the liver by the portal vein before entering the general circulation. Perhaps portal elevations of glucose support taste-to-postingestive consequence learning.

Tordoff & Friedman (1986) provided evidence that portal elevations of glucose may support taste-to-postingestive consequence learning by demonstrating that rats prefer flavours paired with portal infusions of glucose relative to flavours paired with portal infusions of saline. However, it is unclear from their results whether portal elevations of glucose, _in isolation_, can support taste-to-postingestive consequence learning since they paired portal infusions of glucose with flavoured chow (Tordoff & Friedman, 1986). Their protocol cannot rule out the

possibility that one or more of the nutritive consequences of chow contributed to the development of the taste preference.

In experiments 6A and 6B, I test the ability of portal glucose elevations, in isolation, to support taste-to-postingestive consequence learning by pairing flavour cues in non-nutritive saccharin solutions with portal infusions of glucose and saline. In Experiment 7, I replicate the ability of portal infusions of glucose to support a flavour preference when flavour cues are added to nutritive solutions.

EXPERIMENT 6A

This experiment tests the ability of portal elevations of glucose, in isolation, to produce taste-to-postingestive consequence learning. Flavour cues in non-nutritive saccharin solutions are paired with portal infusions of glucose and saline. The infusion rate in this experiment delivers, approximately, the same amount of glucose/infusion as Tordoff & Friedman (1986) used to produce a flavour preference with portal infusions of glucose. If portal glucose elevations, in isolation, support taste-to-postingestive consequence learning, rats in this experiment should prefer the flavour of saccharin paired with portal infusions of glucose. Alternatively, if

portal glucose elevations alone are insufficient to support taste-to-postingestive consequence learning, rats' flavour preferences should be unaffected by portal infusions.

METHOD

Twelve rats served as subjects. Rats weighed 250 to 270 grams at the onset of training and 350 to 510 grams at portal vein catheterization. Rats were food deprived three hours before testing. Water was removed from the cages during the two hour testing period. Testing began three hours into the dark phase of the day-night cyle. Testing was conducted during the dark-phase to ensure that rats would readily consume the saccharin solutions.

The experiment had two phases. In Phase 1, rats were trained to real feed 0.15% (weight/volume) unflavoured saccharin. When their 30 minute intakes of saccharin had stabilized, rats were implanted with portal catheters. Training trials with unflavoured saccharin recommenced the day following portal surgery and continued for seven more days. Then rats' baseline preferences for the flavoured saccharin solutions used in Phase 2 were assessed with a single 30 minute, two-bottle preference test. Exposing rats to these solutions, minimized neophobia in Phase 2 and allowed animals to be divided into two groups, matched for baseline flavour preferences. In the last two days of phase 1, rats were habituated to the infusion procedure. During

habituation trials, rats drank unflavoured saccharin for 30 minutes while connected to the infusion tubing, but nothing was infused.

In Phase 2, rats real fed one flavour of 0.15% saccharin for 30 minutes while receiving a portal infusion of 5% glucose (10 mls/2 hours) and real fed the other flavour of 0.15% saccharin for 30 minutes while receiving a portal infusion of 0.9% saline (10 mls/2 hours). The flavour paired with portal infusions of glucose was counterbalanced. Rats were named for the flavour of saccharin solution that was paired with the glucose infusion. Thus, LEMON rats real fed lemon-flavoured saccharin while receiving portal infusions of glucose; ALMOND rats real fed almond-flavoured saccharin while receiving portal infusions of glucose. The order of infusions was counterbalanced within flavour groups and presented in an ABBA sequence. Rats received four trials of each infusate paired with the appropriate flavour of saccharin. Infusions were two hours long and began 15 minutes after saccharin access. Delaying the onset of the infusion minimized unconditioned suppression of saccharin intake during portal infusions of glucose (Tordoff & Friedman, 1986; Tordoff et al, 1989). Rats were disconnected from the infusion tubing and received chow and water at the end of testing. Chow intake was recorded for the first hour following infusions to determine if glucose

infusions produced an unconditioned suppression of chow intake.

RESULTS

Saccharin consumption during conditioning was collapsed across trials (see Table 1) and then analyzed with a two way ANOVA with Flavour Group (ALMOND vs LEMON rats) and Infusion Condition (glucose infusion or saline infusion) as factors. No significant differences in saccharin intake were detected during infusions ($p>.05$).

Figure 8 shows chow intake in the first hour following portal infusions of glucose or saline. Rats ate approximately the same amount of chow following the first infusion of glucose and saline. With subsequent infusions, rats consumed less chow following glucose infusions. A two way ANOVA, with Trials (1 to 4) and Infusion Condition (glucose vs saline infusion) as factors, indicated that portal infusions of glucose significantly suppressed chow intake [$F(1,11)=6.4$, $p<.03$).

Rats' 30 minute baseline and final preferences for flavours paired with glucose and saline infusions are presented in Figure 9. A two way ANOVA, with Test (baseline preference vs final preference) and Infusion Condition (flavours paired with glucose infusions vs flavours paired with saline infusions) as factors, revealed no significant differences in taste preferences. In particular, rats did

Table 1: Saccharin Consumption during Portal Infusions of Glucose or Saline (Experiment 6A)

		SACCHARIN CONSUMPTION (mls) during	
Flav. Group	No. of Sjs	Glu. Infusions	Sal. Infusions
ALMOND	4	6.0	6.8
LEMON	8	7.4	7.0

[Flav Group = flavour group, Glu. = glucose, Sal. = saline, No. of Sjs = number of subjects]

Figure 8: Chow intake (+/- SEM) in the first hour following two hour portal infusions (10mls/2 hours) of 5% glucose or 0.9% saline. GLU INFUSION = intake following portal infusions of glucose, SAL INFUSION = intake following portal infusions of saline.

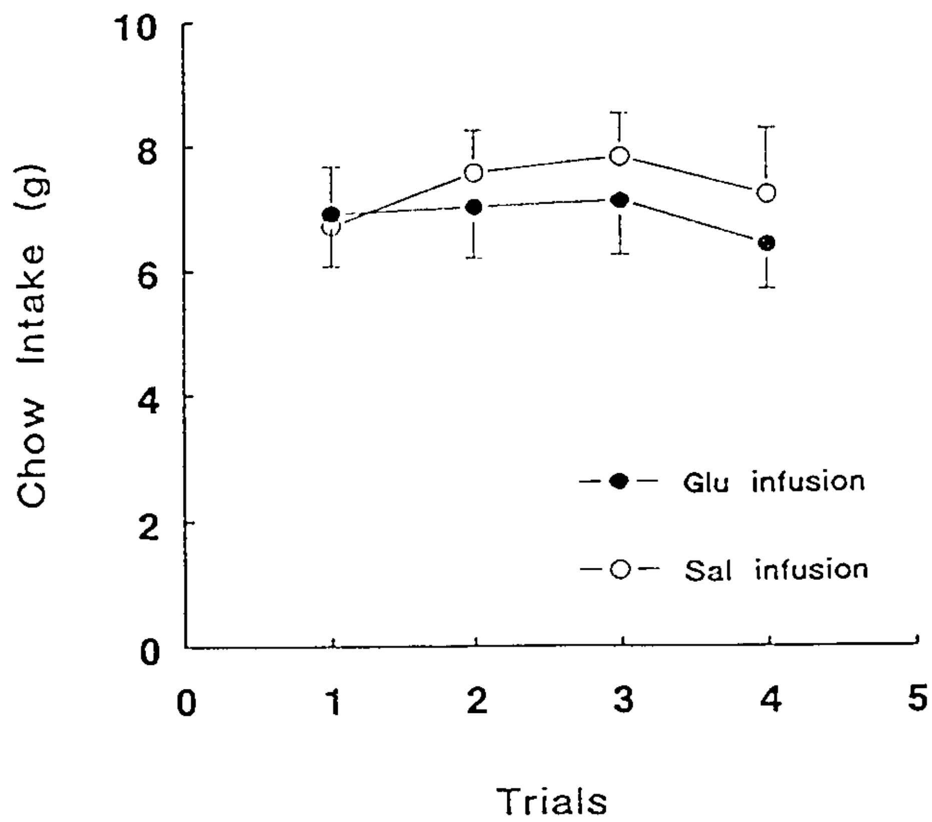
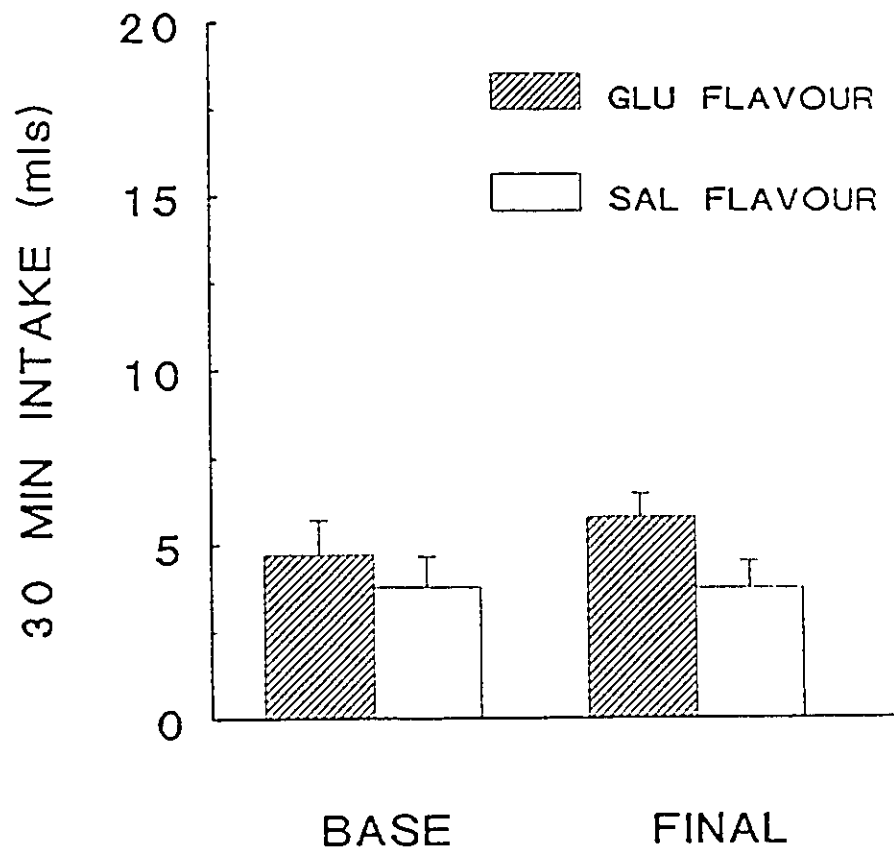

10
8
6
4
2
0
Chow Intake (g)
Glu infusion
Sal infusion
0
1
2
3
4
5
Trials

Figure 9: 30 minute baseline (BASE) and final (FINAL) preferences (+/- SEM) for saccharin flavours previously paired with portal infusions of glucose or saline. GLU FLAVOUR = saccharin flavours previously paired with portal infusions of glucose, SAL FLAVOUR = saccharin flavours previously paired with portal infusions of saline.



20
15
10
5
0
30 MIN INTAKE (mls)
GLU FLAVOUR
SAL FLAVOUR
BASE
FINAL

not develop a preference for the flavour of saccharin that had been paired with glucose infusions (Test x Infusion Condition $F(1,11)=1.2$, NS). Rats also did not demonstrate a preference when the simple presence or absence of a preference was assessed. Seven out of twelve rats preferred the flavour paired with portal infusions of glucose at baseline and eight rats preferred the same flavour after conditioning.

DISCUSSION

This experiment demonstrates that portal infusions of glucose can suppress eating, an effect that has been reported before (eg. Tordoff & Friedman, 1986; Tordoff et al, 1989). In this experiment, rats ate less chow during the first hour following a glucose infusion than following a saline infusion.

The critical question for this experiment, however, was whether portal infusions of glucose could support a flavour preference, a behavioral marker for taste-to-postingestive consequence learning. This experiment demonstrates that pairing a distinctively flavoured saccharin solution with portal infusions of glucose does not produce a flavour preference. The results of this study contrast with those of Tordoff & Friedman (1986) who demonstrated, using similar infusion parameters, that rats prefer the flavour of chow that had been paired with portal

infusions of glucose. However, the current experiment tests whether portal elevations, in isolation, can support a taste preference. Tordoff & Friedman (1986) added their taste cues to nutritive chow and their protocol cannot rule out the possibility that one or more of the nutritive consequences of chow contributed to the taste preference they observed.

The inability of portal infusions of glucose, in this experiment to establish a flavour preference suggests that portal elevations of glucose, produced by infusion parameters in this study, do not support taste-to-postingestive consequence learning. In Experiment 6B, I explore whether increasing the amount of glucose infused into the portal vein can produce a taste preference.

EXPERIMENT 6B

Experiment 6A demonstrates that portal infusions of 0.5 g glucose/2 hours do not support taste-to-postingestive consequence learning. This experiment tests whether portal infusions of 1.0 g glucose/2 hours support taste-to-postingestive consequence learning. Tordoff et al (1989) demonstrated that infusions of glucose into the portal vein at 1.0 g glucose/2 hours produce portal glucose values that approximate those recorded, by others, during real feeding

(Langhans et al, 1982; Niewoehner & Nuttal, 1989; Smadja, Morin, Ferré & Girard, 1988; Strubbe & Steffans, 1977).

METHOD

Eight rats served as subjects. Rats weighed 260 to 280 grams at the onset of training and 350 to 390 grams at portal vein catheterization.

The protocol in this experiment was identical to that used in Experiment 6A except that 10% (w/v) glucose, not 5% (w/v) glucose, was infused into rats' portal veins.

RESULTS

Saccharin consumption during conditioning was collapsed across trials (see Table 2) and then analyzed with a two way ANOVA with Flavour Group (ALMOND rats vs LEMON rats) and Infusion Condition (glucose infusion or saline infusion) as factors. No significant differences in saccharin intake were detected during infusions ($p>.05$).

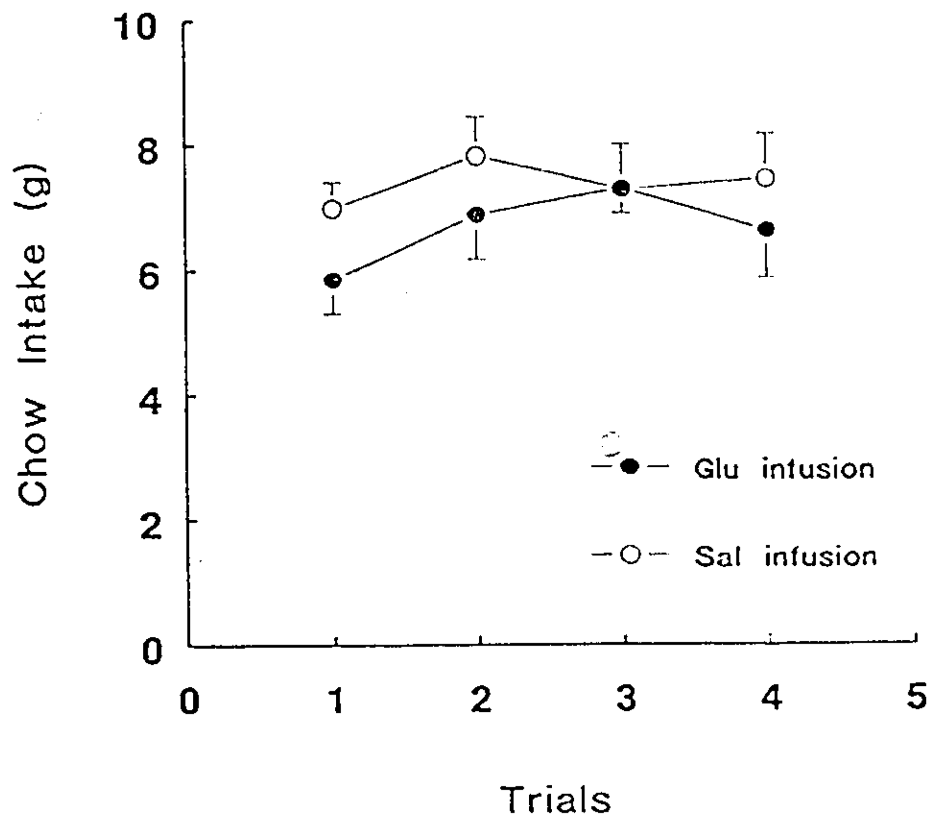
Figure 10 shows chow intake in the first hour following portal infusions of glucose or saline. Initially, rats ate less chow following glucose infusions. However, a two way ANOVA, with Trials (1 to 4) and Infusion Condition (glucose infusion vs saline infusion) as factors, indicated portal infusions of glucose did not significantly suppress chow intake ($F(1,7)=2.07$, NS)].

Table 2: Saccharin Consumption during Portal Infusions of Glucose or Saline (Experiment 6B)

		SACCHARIN CONSUMPTION (mls) during	
Flav. Group	No. of Sjs	Glu. Infusions	Sal. Infusions
ALMOND	4	11.8	10.2
LEMON	4	11.3	10.9

[Flav Group = flavour group, Glu. = glucose, Sal. = saline, No. of Sjs = number of subjects]

Figure 10: Chow intake (+/- SEM) in the first hour following two hour portal infusions (10mls/2 hours) of 10% glucose or 0.9% saline. GLU INFUSION = intake following portal infusions of glucose, SAL INFUSION = intake following portal infusions of saline.



Chow Intake (g)
10
8
6
4
2
0
0
1
2
3
4
5
Trials
Glu infusion
Sal infusion

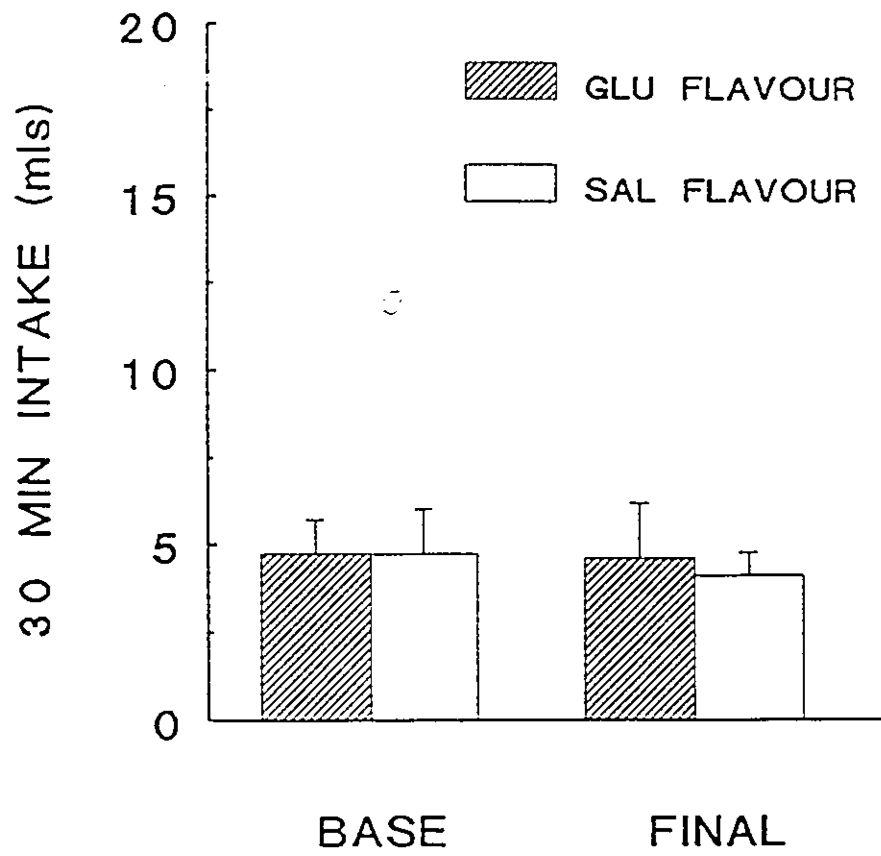
Rats' 30 minute baseline and final preferences for flavours paired with portal infusions of glucose and saline are presented in Figure 11. A two way ANOVA, with Test (base preference vs final preference) and Infusion Condition (flavours paired with glucose infusions vs flavours paired with saline infusions) as factors, indicated that rats did not develop a preference for saccharin paired with glucose infusions (Test x Infusion Condition $F(1,7)<1$, NS). Rats also did not demonstrate a preference when the simple presence or absence of a preference was assessed. Four out of eight rats preferred the flavour paired with portal infusions of glucose at baseline, and after conditioning.

DISCUSSION

This experiment replicates the inability of portal infusions of glucose to support a taste preference (Experiment 6A). Since portal infusions of glucose in this study delivered twice the amount of glucose as Experiment 6A, it is unlikely that I failed to detect taste-to-postingestive consequence learning in Experiment 6A because the infusion parameter was too low. Rather, the results of this experiment and Experiment 6A suggest that portal elevations of glucose, in isolation, do not support taste-to-postingestive consequence learning.

This experiment fails to replicate the ability of portal infusions of glucose to significantly suppress chow

Figure 11: 30 minute baseline (BASE) and final (FINAL) preferences (+/- SEM) for saccharin flavours previously paired with portal infusions of glucose and saline. GLU FLAVOUR = saccharin flavours previously paired with portal infusions of glucose, SAL FLAVOUR = saccharin flavours previously paired with portal infusions of saline.



20
15
10
5
0
30 MIN INTAKE (mls)
GLU FLAVOUR
SAL FLAVOUR
BASE
FINAL

intake (Experiment 6A). However, both experiments 6A and 6B produced similar mean decreases in chow intake (Expt 6A (X=-.48 g); Expt 6B (X=-.72 g)).

EXPERIMENT 7

Experiments 6A and 6B suggest that portal elevations of glucose, in isolation, cannot support taste-to-postingestive consequence learning. In this experiment, I assess whether portal elevations of glucose support taste-to-postingestive consequence learning when taste cues are presented in nutritive solutions. Using a between-subject design, portal infusions of glucose are either paired with taste cues added to nutritive glucose solutions or non-nutritive saccharin solutions. If taste cues must be added to a nutritive solution for portal infusions of glucose to support taste-to-postingestive consequence learning, rats which experience taste cues in glucose solutions should demonstrate taste-to-postingestive consequence learning; rats which experience taste cues in saccharin solutions should not.

METHOD

Fourteen rats served as subjects. Rats weighed 310 to 410 grams at the start of training and 400 to 470 grams at portal vein catheterization. Rats were given ad lib

access to food and water before all trials. Water and food was removed from the cages during the two hour testing period. Testing began at the onset of the dark phase of the light-dark cycle to replicate the conditions of Experiments 6A & 6B.

Initially, rats were divided into two groups named for the solution they would consume throughout the experiment. GLUCOSE rats consumed an 18% (w/v) glucose solution; SACCHARIN rats consumed 0.15% (w/v) saccharin solution.

The experiment had two phases. In Phase 1, rats were trained to drink the appropriate unflavoured saccharin or glucose solution. When their 30 minute intakes had stabilized, rats were implanted with portal catheters. Training trials with the appropriate unflavoured solution recommenced the day following portal surgery and continued for seven more days. Next rats' baseline preferences for the flavours of glucose or saccharin solutions used in Phase 2 were assessed with a single 30 minute, two-bottle preference test. Exposing rats to these flavoured solutions minimized neophobia in Phase 2 and allowed GLUCOSE and SACCHARIN rats to be subdivided into two groups, matched for baseline flavour preferences. In the last two days of Phase 1, all rats were habituated to the infusion procedure. During habituation trials, rats were connected to the infusion tubing but nothing was infused. During habituation

trials, SACCHARIN rats had 30 minute access to unflavoured saccharin; GLUCOSE rats had 30 minute access to unflavoured glucose.

In Phase 2, SACCHARIN rats real fed one flavour of 0.15% saccharin for 30 minutes while receiving a portal infusion of 10% glucose (10 mls/2 hours) and real fed the other flavour of 0.15% saccharin while receiving a portal infusion of 0.9% saline (10 mls/2 hours). The procedure was identical for GLUCOSE rats except that portal infusions of glucose and saline were paired with distinctively flavoured 18% glucose solutions. The flavour (almond or lemon) paired with glucose infusions was counterbalanced within each solution group (GLUCOSE, SACCHARIN). Rats were named for the flavour paired with portal infusions of glucose. Thus, ALMOND rats consumed an almond-flavoured solution during portal infusions of glucose. LEMON rats consumed a lemon-flavoured solution during portal infusions of glucose. The order of infusions was counterbalanced within flavour subgroups and presented in an ABBA sequence. Rats received six trials of each infusate paired with the appropriate flavour of a saccharin or glucose solution. Infusions were two hours long and began at the onset of access to flavoured saccharin or glucose solutions. Rats were disconnected from the infusion tubing and received chow and water at the end of testing. Chow intake was recorded for the first hour

following infusions to determine if glucose infusions produced an unconditioned suppression of chow intake.

RESULTS

All GLUCOSE and SACCHARIN rats completed 3 hours of preference testing but one rat from each group failed to complete the final hour of preference testing; one GLUCOSE rat overturned a solution bottle and the intake of one flavoured saccharin solution was not recorded in the final hour for one SACCHARIN rat.

GLUCOSE rats

Oral glucose solution consumption during conditioning was collapsed across trials (see Table 3) and then analyzed with a two way ANOVA with Flavour Group (ALMOND rats vs LEMON rats) and Infusion Condition (glucose infusion or saline infusion) as factors. No significant differences in glucose solution intake were detected during infusions ($p>.05$).

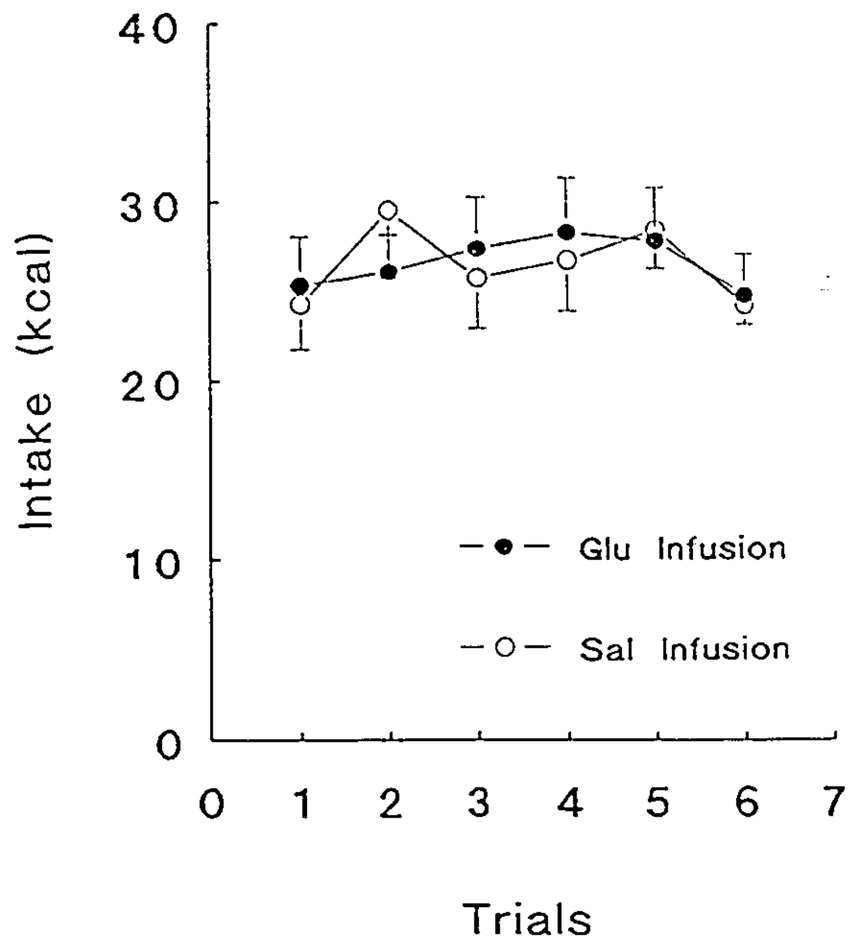
To assess whether glucose infusions produced an unconditioned suppression of intake, rats' solution intake during infusions and their chow intake following infusions were converted to kilocalories (kcal), (1 g glucose = 3.6 kcal; 1 g chow = 3.3 kcal). Figure 12 shows GLUCOSE rats' kcal intake. A two way ANOVA, with Trials (1 to 6) and Infusion Condition (glucose infusion vs saline infusion) as

Table 3: Oral Glucose Consumption during Portal Infusions of Glucose or Saline (Experiment 7, GLUCOSE rats)

		ORAL GLUCOSE CONSUMPTION (mls) during	
Flav. Group	No. of Sjs	Glu. Infusions	Sal. Infusions
ALMOND	3	13.3	11.8
LEMON	4	11.7	11.1

[Flav Group = flavour group, Glu. = glucose, Sal. = saline, No. of Sjs = number of subjects]

Figure 12: Kilocalorie (kcal) intake (+/- SEM) for GLUCOSE rats in the first hour following two hour portal infusions (10mls/2 hours) of 10% glucose or 0.9% saline. GLU INFUSION = kcal intake following portal infusions of glucose, SAL INFUSION = kcal intake following portal infusions of saline.


40
30
20
10
0
Intake (kcal)
Glu Infusion
Sal Infusion
0
1
2
3
4
5
6
7
Trials

factors, indicated that portal infusions of glucose had no effect on GLUCOSE rats kcal intake [F(1,6)<1, NS].

GLUCOSE rats' 30 minute baseline and final preferences for flavours paired with glucose and saline infusions are presented in Figure 13. A two way ANOVA, with Test (baseline preference vs final preference) and Infusion Condition (flavours paired with glucose infusions vs flavours paired with saline infusions) as factors, revealed no significant preference for the flavour of paired with glucose infusions (Test x Infusion Condition $F(1,6)=2.6$, $p=.16$).

Preferences for flavours paired with portal infusions of glucose and saline over a four hour period are presented in Figure 14 for the six GLUCOSE rats that completed testing. As Figure 14 indicates, GLUCOSE rats preferred the flavour that had previously been paired with portal infusions of glucose throughout testing. GLUCOSE rats' preferences were tested with matched t-tests at 60, 120, 180 and 240 minutes. A significant preference (71%) was detected at 240 minutes ($t(5)=-3.1$, $p<.03$). Also, all rats (N=6) preferred the flavour paired with portal infusions of glucose from minutes 180 to 240.

<u>SACCHARIN rats</u>

Saccharin solution consumption during conditioning was collapsed across trials (see Table 4) and then analyzed

Figure 13: 30 minute baseline (BASE) and final (FINAL) preferences (+/- SEM) for GLUCOSE rats. GLU FLAVOUR = glucose flavours paired with portal infusions of glucose, SAL FLAVOUR = glucose flavours paired with portal infusions of saline.

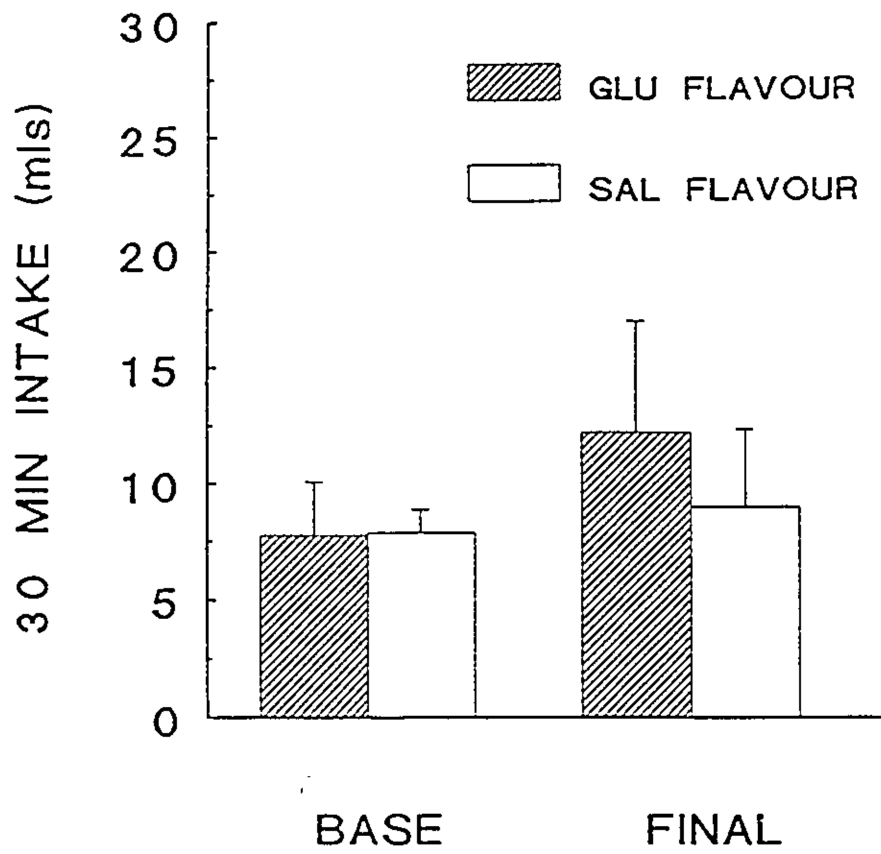

30 MIN INTAKE (mls)
30
25
20
15
10
5
0
GLU FLAVOUR
SAL FLAVOUR
BASE
FINAL

Figure 14: GLUCOSE rats cumulative (15 - 240 minutes) preferences (+/- SEM) for glucose flavours previously paired with portal infusions of glucose or saline. GLU FLAVOUR = glucose flavours previously paired with portal infusions of glucose, SAL FLAVOUR = glucose flavours previously paired with portal infusions of saline.

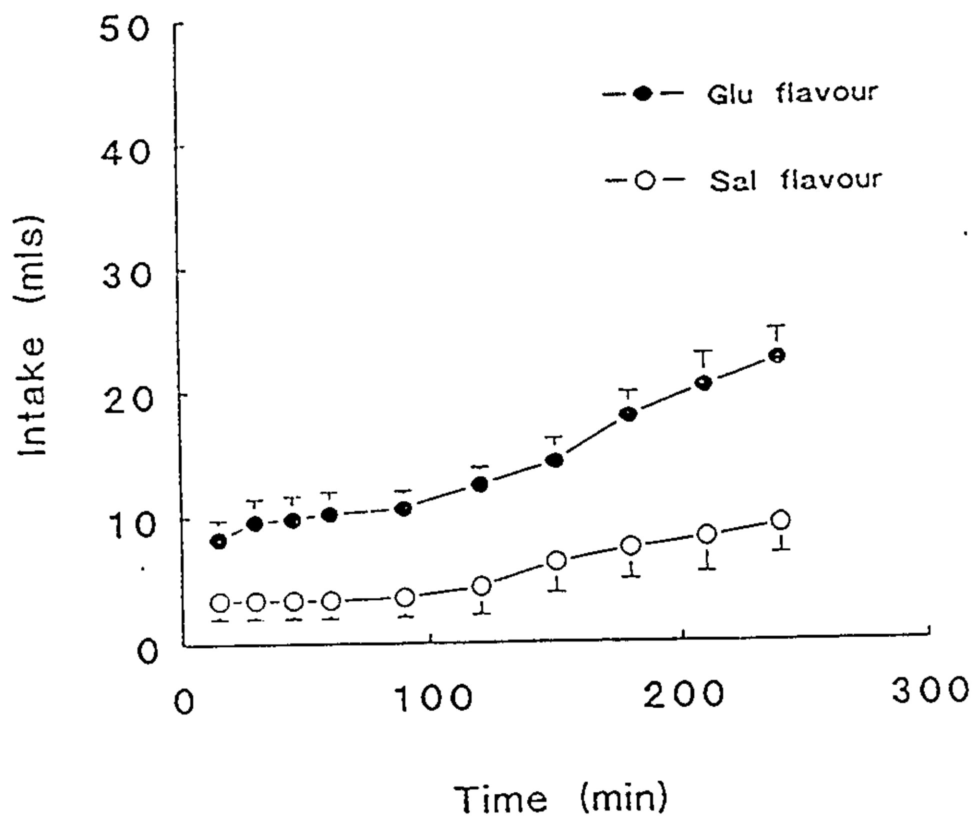

50
40
30
20
10
0
Intake (mls)
0
100
200
300
Time (min)
Glu flavour
Sal flavour

Table 4: Saccharin Consumption during Portal Infusions of Glucose or Saline (Experiment 7, SACCHARIN rats)

		SACCHARIN CONSUMPTION (mls) during	
Flav. Group	No. of Sjs	Glu. Infusions	Sal. Infusions
ALMOND	3	23.9	20.58
LEMON	4	9.8	11.9

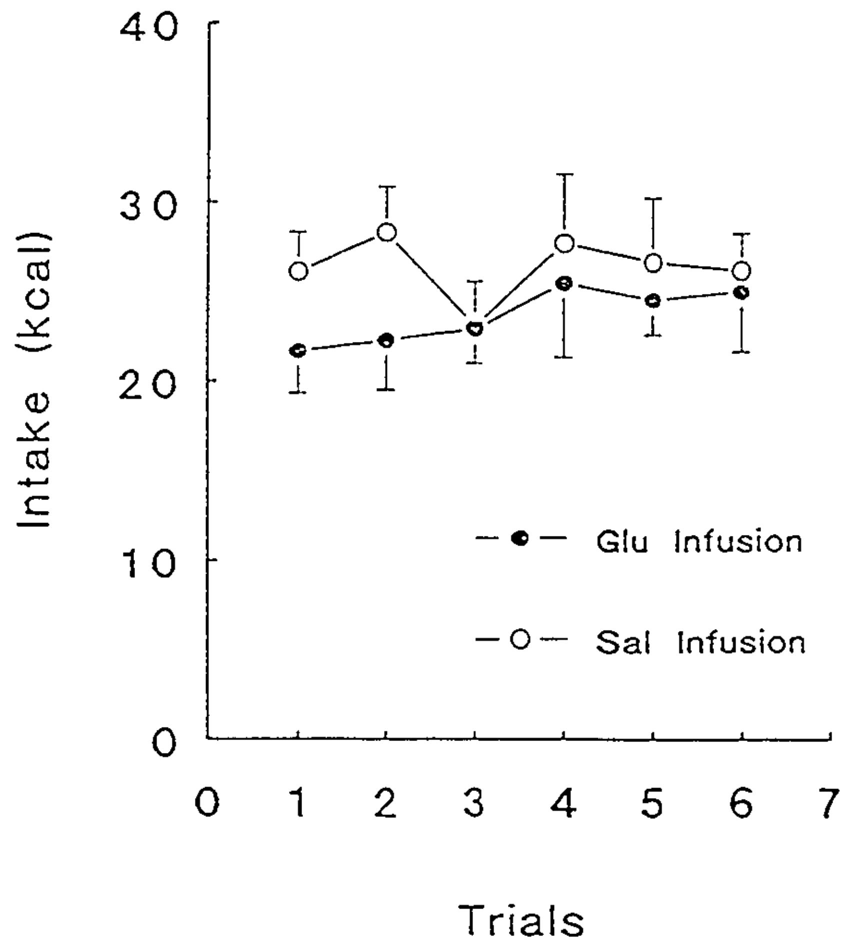
[Flav Group = flavour group, Glu. = glucose, Sal. = saline, No. of Sjs = number of subjects]

with a two way ANOVA with Flavour Group (ALMOND rats vs LEMON rats) and Infusion Condition (glucose infusion or saline infusion) as factors. ALMOND rats consumed slightly more saccharin solution during glucose infusions (23.9 mls vs 20.58 mls) while LEMON rats consumed slightly more saccharin solution during saline infusions (9.8 mls vs 11.9 mls), producing a significant Group x Infusion interaction ($F(1,5)=10.8$, $p<.03$).

To be consistent with GLUCOSE rats, the chow intake of SACCHARIN rats was converted to kcal intake (saccharin intake during infusions was non-nutritive and contributed no kcal). Figure 15 shows SACCHARIN rats' kcal intake. Although glucose infusions suppressed chow intake in SACCHARIN rats, a two way ANOVA, with Trials (1 to 6) and Infusion Condition (glucose infusion vs saline infusion) as factors, indicated that this suppression was not significant ($F(1,6)=4.4$, $p<.078$)].

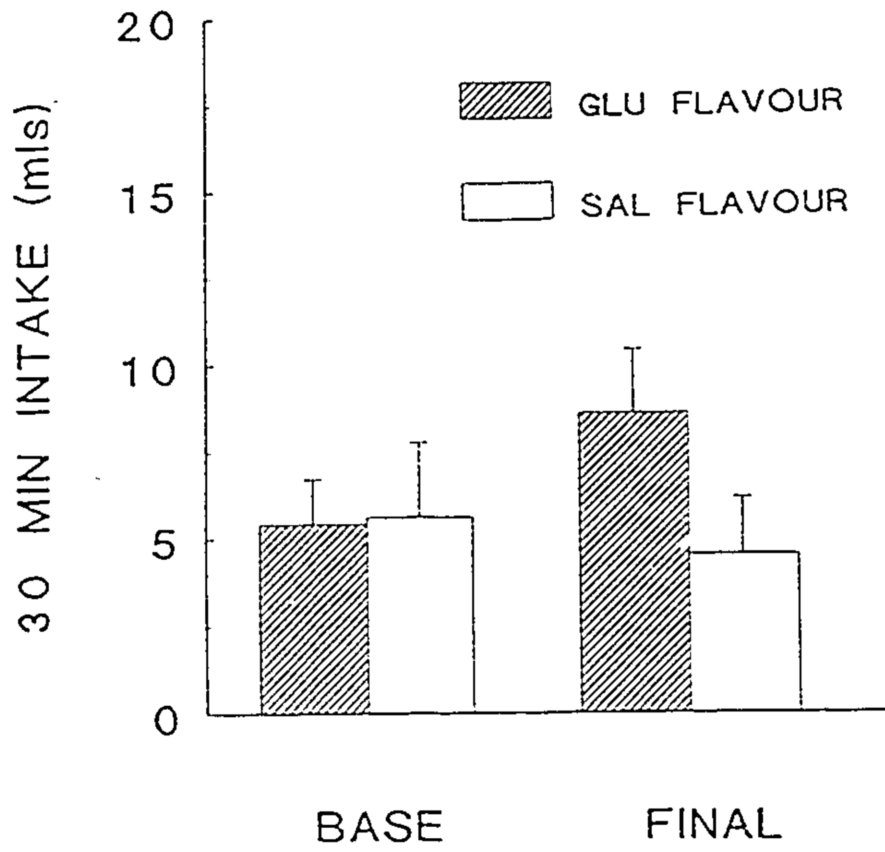
SACCHARIN rats' 30 minute baseline and final preferences for flavours paired with glucose and saline infusions are presented in Figure 16. A two way ANOVA, with Test (base preference vs final preference) and Infusion Condition (flavour paired with glucose infusions vs flavour paired with saline infusions) as factors, revealed no significant preference for the flavour that had been paired with glucose infusions (Test x Infusion Flavour $F(1,6)<1$, NS).

Figure 15: Kilocalorie (kcal) intake (+/- SEM) for SACCHARIN rats in the first hour following two hour portal infusions (10mls/2 hours) of 10% glucose or 0.9% saline. GLU INFUSION = kcal intake following portal infusions of glucose, SAL INFUSION = kcal intake following portal infusions of saline.



40
30
20
10
0
Intake (kcal)
0
1
2
3
4
5
6
7
Trials
Glu Infusion
Sal Infusion

Figure 16: 30 minute baseline (BASE) and final (FINAL) preferences (+/- SEM) for SACCHARIN rats. GLU FLAVOUR = saccharin flavours previously paired with portal infusions of glucose, SAL FLAVOUR = saccharin flavours previously paired with portal infusions of saline.


30 MIN INTAKE (mls)
20
15
10
5
0
GLU FLAVOUR
SAL FLAVOUR
BASE
FINAL

Preferences for saccharin flavours paired with portal infusions of glucose and saline over four hours are presented in Figure 17 for the six SACCHARIN rats that completed testing. Although SACCHARIN rats showed a slight preference for the flavour paired with portal infusions of glucose during testing, matched t-tests at 60, 120, 180 and 240 minutes failed to reveal a significant preference ($p>.05$). Also, at 240 minutes only three of six rats preferred the flavour that had been paired with portal infusions of glucose.

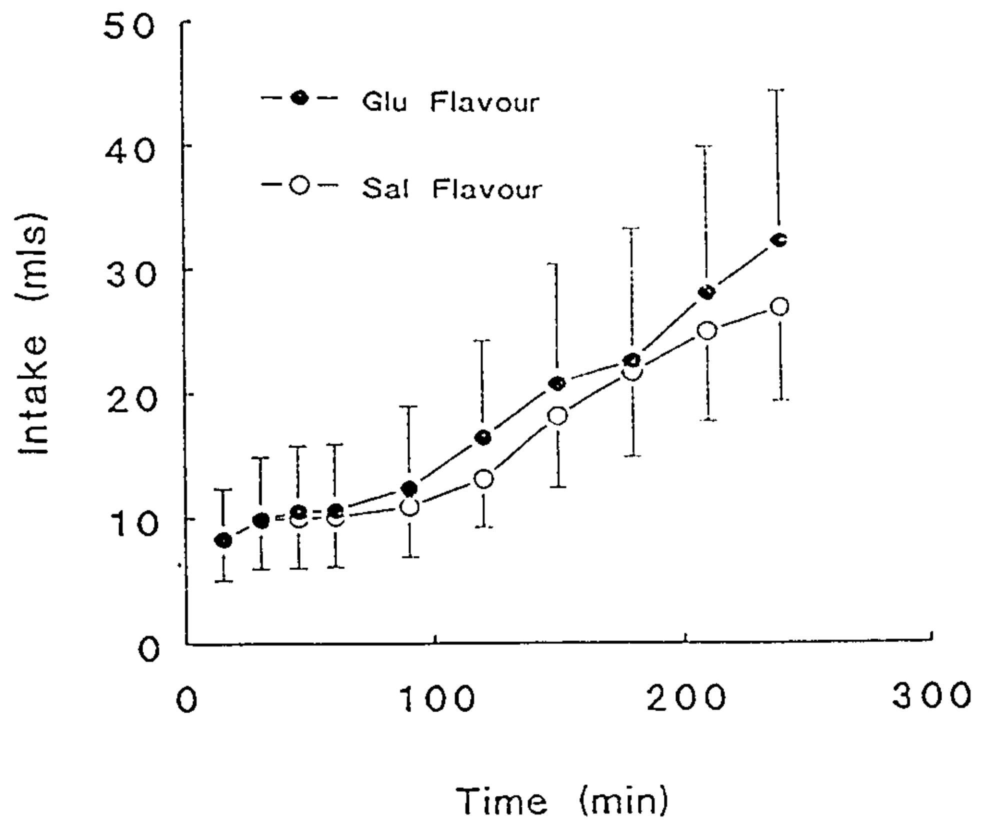
GLUCOSE and SACCHARIN rats

The presence of a preference for flavours paired with portal infusions of glucose at 240 minutes was compared in GLUCOSE and SACCHARIN rats using a X^2 test (see Table 5). A significant relationship existed between the development of a preference and the nutritive value of the solution ($X^2=4.0$, $p=.023$). Visual inspection of Table 5 reveals that GLUCOSE rats developed a preference for flavours paired with portal infusions of glucose; SACCHARIN rats did not.

DISCUSSION

This experiment replicates the ability of portal infusions of glucose to support a 4 hour flavour preference when portal infusions of glucose are paired with flavours in nutrient (Tordoff & Friedman, 1986).

Figure 17: SACCHARIN rats cumulative (15 - 240 minutes) preferences (+/- SEM) for flavours previously paired with portal infusions of glucose or saline. GLU FLAVOUR = saccharin flavours previously paired with portal infusions of glucose, SAL FLAVOUR = saccharin flavours previously paired with portal infusions of saline.



Glu Flavour
Sal Flavour
Intake (mls)
0
10
20
30
40
50
0
100
200
300
Time (min)

Table 5: Relationship between Solution Group (GLUCOSE vs SACCHARIN rats) and a CS+ preference (Experiment 7).

	PREFERENCE	NO PREFERENCE	
GLUCOSE rats	6	0	6
SACCHARIN rats	3	3	6
	9	3	12

[Rats were assigned a CS+ preference if CS+ intake > CS- intake]

This study also provides evidence that presenting flavour cues in nutrient may be a <u>necessary</u> condition for portal infusions of glucose to support a flavour preference. This experiment demonstrates that GLUCOSE rats which received portal infusions of glucose paired with flavoured, nutritive glucose solutions, established a flavour preference while SACCHARIN rats which received portal infusions of glucose paired with flavoured, non-nutritive saccharin solutions, did not.

It is unclear why presenting flavour cues in nutrient facilitates the establishment of a preference. However, there are at least two possibilities. First, nutrient when absorbed will, in and of itself, raise portal levels of glucose. Perhaps flavour cues must be added to nutrient because portal elevations of glucose only support taste preferences at suprathreshold levels. Second, nutrient provides chemostimulation to the entire gastrointestinal tract. Perhaps portal elevations of glucose only support taste preferences when they are potentiated by signals arising from other sites in the gastrointestinal tract.

CHAPTER VI. GENERAL DISCUSSION

This thesis assesses the relevance of plasma and portal elevations of glucose as consequences in taste-to-postingestive consequence learning.

Experiments in this thesis demonstrate that plasma glucose elevations do not support taste-to-postingestive consequence learning. When I paired a taste with a plasma glucose profile, characteristic of real feeding, rats did not demonstrate taste-to-postingestive consequence learning, regardless of whether a sham feeding or preference probe was used to assess learning (Experiment 3). Also, glucose infusions during sham feeding were behaviorally silent when animals were subsequently switched to real feeding (Experiment 4). Rats behaved on the first real feeding trial, after a long history of sham feeding during glucose infusions, as if they had only been sham feeding.

The conclusion that plasma glucose elevations do not support taste-to-postingestive consequence learning is consistent with the work of others who assessed the ability of plasma glucose elevations to establish a taste preference (Revusky et al, 1971; Tordoff & Friedman, 1986). However, I extend their work by testing for the presence of taste-to-

postingestive consequence learning with a different behavioral probe: namely, taste-specific suppression of sham feeding intake. And, most importantly, I demonstrate that my manipulation of plasma glucose mimics the time course of plasma glucose following a meal.

In the course of investigating the relevance of plasma glucose in taste-to-postingestive consequence learning, I noted, as have others (Berthoud, 1984; Sclafani & Nissenbaum, 1985), that sham feeding produces elevations in plasma glucose. These elevations must reflect the absorption of nutrient since I demonstrated that plasma radioactivity increased when rats sham fed a radiolabelled solution (Experiment 5).

Although not the primary focus of this thesis, two separate findings indicate that plasma glucose does not contribute to meal termination. First, I recorded similar levels of plasma glucose during real and sham feeding at a time (15 minutes after meal initiation) when the real feeding rat had stopped eating but the sham feeding rat continued to eat at a high rate (Experiment 1). Second, infusions of 10% glucose, at a rate that produced plasma glucose levels similar to those of real feeding (Experiment 2), did not decrease sham feeding intake (Experiment 3). Although this conclusion may not generalize beyond the experimental conditions used here, the conclusion that plasma glucose does not control meal termination is

consistent with a host of other studies that fail to demonstrate a significant effect of elevated plasma glucose on intake (eg. Baile, Zinn & Mayer, 1971; Janowitz, Hanson & Grossman, 1949; Stephens & Baldwin, 1974; Strubbe & Steffens, 1976; Tordoff & Friedman, 1986 but see Mather et al, 1978; Smith, 1966).

Three conclusions were drawn from experiments that assessed the relevance of portal elevations of glucose in taste-to-postingestive consequence learning. First, portal elevations of glucose, _in isolation_, do not appear to support taste-to-postingestive consequence learning. Rats that received portal infusions of glucose paired with flavoured, non-nutritive saccharin solutions did not develop a flavour preference (Experiments 6A, 6B & 7). Second, presenting flavour cues in nutrient may be a necessary condition for portal elevations of glucose to support a flavour preference. GLUCOSE Rats which received portal infusions of glucose paired with flavoured, nutritive glucose solutions, established a flavour preference; SACCHARIN rats which received portal infusions of glucose paired with flavoured, non-nutritive saccharin solutions, did not (Experiment 7). Third, portal infusions of glucose may generate different signals for the suppression of food intake and the establishment of a taste preference since these effects can be dissociated. In Experiment 6A, portal

infusions of glucose suppressed food intake but did not produce a taste preference. In Experiment 7, portal infusions of glucose produced a preference in GLUCOSE rats but did not suppress their food intake.

It is unclear whether embedding flavour cues in nutritive solutions facilitates taste-to-postingestive consequence learning by increasing the magnitude of portal elevations of glucose or by stimulating other sites in the gastrointestinal tract.

If flavour cues must be added to nutrient merely to raise portal glucose to suprathreshold levels then, contrary to the results of the saccharin experiments (Experiments 6A, 6B & 7), high levels of portal glucose, in isolation, may be sufficient to establish taste-to-postingestive consequence learning. A direct test of this hypothesis would be to match the quantity of glucose delivered to the portal veins of GLUCOSE and SACCHARIN rats. Although doubling the amount of glucose infused into the portal vein in Experiment 6B did not establish a flavour preference when flavour cues were presented in saccharin solutions, matching the amount of glucose delivered to the portal vein in GLUCOSE rats and SACCHARIN rats would require that SACCHARIN rats receive substantially more glucose than delivered in any experiment in this thesis. If one assumes that all glucose ingested orally is absorbed into the portal vein during the 2 hour infusion period, SACCHARIN rats would require a further

doubling of the amount of glucose infused to receive approximately the amount of glucose delivered to the portal vein of GLUCOSE rats. (GLUCOSE rats consumed an average of 12.3 mls of 18% glucose in Experiment 7 or 2.2 grams of glucose).

One could test the hypothesis that portal elevations of glucose only support a taste preference when potentiated by other gastrointestinal (GI) signals by selectively attenuating putative GI signals and observing the effect this manipulation has on the ability of portal glucose elevations to support a taste preference. For example, some have suggested that CCK secretion is a putative consequence in taste-to-postingestive consequence learning (Mehiel & Bolles, 1988; Perez & Sclafani, 1991). One could test the relevance of portal glucose elevations and CCK as a compound consequence in taste-to-postingestive consequence learning by selectively antagonizing peripheral CCK receptors during portal infusions of glucose.

To conclude, this thesis demonstrates that plasma glucose elevations do not support taste-to-postingestive consequence learning and that portal elevations of glucose may only support taste-to-postingestive consequence learning under defined conditions.

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