CCK, Dietary Variety, and Food Intake in Rats

by

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Abstract

Among the environmental factors that can affect food intake is the extent of dietary variety available in the environment. Numerous studies have demonstrated that variety in a meal can increase the amount of food consumed in humans, rats, and other species. A physiological mechanism that has been demonstrated to affect food intake is the gut peptide cholecystokinin (CCK) which is released from the upper small intestine during the ingestion of food. Peripherally administered CCK has a robust inhibitory effect on the intake of a single-food meal. Thus, dietary variety and CCK both affect meal size, with dietary variety increasing intake and CCK decreasing intake. This raises the question of how dietary variety and CCK might interact to affect meal size. Previous studies of CCK’s effects have focused on situations in which only one food was available for consumption. However, in an animal’s natural environment it would frequently occur that the animal would come across a number of foods either simultaneously or in quick succession, thus providing the animal access to a variety of foods during a meal. Accordingly, the effect of CCK on food intake in single-food and multiple-food meals was examined. It was found that food intake was greater in multiple-food than in single-food meals provided that foods in the multiple-food meal were presented either simultaneously or in increasing order of preference. When foods in the multiple-food meal were presented in decreasing order of preference, intake was similar to that observed in single-food meals. In addition, it was found that CCK inhibited food intake in a dose-dependent manner, and that its effects on food intake were similar regardless of meal type. Therefore, the inhibitory effects of CCK were not diminished when a variety of foods were available for consumption. Furthermore, the finding that CCK did not differentially affect the
intake of the two types of meals does not provide support for the recent-foods hypothesis which postulates that CCK decreases food intake by reducing the palatability of only recently consumed foods. However, it is consistent with the all-foods hypothesis, which predicts that CCK reduces food intake by decreasing the palatability of all foods.

The 600 μg/kg dose of the CCK\textsubscript{A}-antagonist lorglumide significantly antagonized the inhibitory effect of exogenous CCK on food intake, and the magnitude of this effect was similar for both types of meal. These results suggest that exogenous CCK inhibits food intake through the activation of CCK\textsubscript{A} receptors. However, when administered by itself, the 600μg/kg dose of lorglumide did not increase food intake in either single-food or multiple-food meals, suggesting that peripheral endogenous CCK may not play a major role in the control of food intake.
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Stefan Brudzynski was also of tremendous help in aiding me in the preparation of my CCK-antagonist. Without his expertise the undertaking of preparing the solution would have been much more daunting.

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List of Abbreviations

CCK = cholecystokinin
MC = milk chocolate
PB = peanut butter diet
SC = shortcake cookie
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Introduction

A number of environmental (as reviewed by De Castro, 1996) and physiological factors (as reviewed by Campfield, 1997; and by De Castro, 1996) have been implicated in the control of eating behaviour. The focus of this thesis will be on how food intake is affected by the interaction of dietary variety, an environmental factor, and the gut peptide cholecystokinin (CCK), a physiological factor.

Dietary variety is one of a number of environmental factors that have been shown to affect food intake. Numerous studies have demonstrated that variety in a meal increases the amount of food consumed by humans, rats, and other species (DiBattista & Sitzer, 1994; Rolls, B.J., Rowe, Rolls, Kingston, Megson, & Gunary, 1981; Rolls, B.J., Van Duijvenvoorde & Rowe, 1983; Treit, Spetch, & Deutsch, 1983). The gut peptide, CCK, which is released from the upper small intestine during the ingestion of food, is one of a number of physiological factors that have been shown to be involved in the control of food intake (as reviewed by Smith & Gibbs, 1998). Peripherally administered CCK has a robust inhibitory effect on food intake in single-food meals (Gibbs, Young, & Smith, 1973; Le Sauter, Goldberg, & Geary, 1988; Moran, Baldessarini, Salorio, Lowery, & Schwartz, 1997; Moran, Katz, Plata-Salaman, & Schwartz, 1998; West, Fey, & Woods, 1984). However, the effect of CCK on food intake in multiple-food meals has not been examined. Of course, in their natural environment, animals may experience dietary variety by coming across a number of foods either simultaneously or in quick succession. Accordingly, in this thesis, the effects of dietary variety and CCK on food intake in rats will be examined, and any interactions that may occur between these two factors will be observed.
The Role of Dietary Variety in Food Intake

There are a number of environmental factors that can influence eating behavior, including social environment, noise, weather (as reviewed by De Castro, 1996), and variety in the meal (DiBattista & Sitzer, 1994; Rolls, B.J., et al. 1983; Treit et al., 1983; Rolls, B.J., Rowe et al., 1981). The effects of dietary variety on food intake will be examined further.

The Effects of Dietary Variety on Food Intake

Dietary variety has been shown to lead to increased food intake in a number of species, including rats, hamsters, and humans (DiBattista & Sitzer, 1994; Rolls, B.J., Rowe et al., 1981; Treit et al., 1983). For example, Treit et al. (1983) used a within-group design with rats having access at various times to single-food and multiple-food meals. The food in both the single-food and multiple-food meals was powdered rat chow containing an added flavour (lemon, maple, mint, and table salt). The single-food meal was presented on four occasions, and then the multiple-food meal was presented on four occasions. Rats received four consecutive courses in each type of meal. During the single-food meal, the four 30-min courses were flavoured the same, whereas in the multiple-food meal each course had a different flavour. During the last three courses, non-deprived rats ate approximately 45% more food (grams) during the multiple-food meal than during the single-food meals (Treit et al., 1983).

Rolls, B.J. et al. (1983) provided rats with single-food and multiple-food meals. Their research differed from the experiment of Treit et al. (1983) in that the foods used (cheese crackers, cookies, and cooking chocolate) differed from each other not only in flavour, but also in nutrient composition and palatability. The meals consisted of three 40-min courses. In the
multiple-food meals, the three foods were presented either simultaneously (i.e. all three foods during each course) or successively (i.e. a different food during each course). Rats consumed on average 26% more energy in the multiple-food meals than in the single-food meals, again supporting the idea that variety can lead to increased food consumption. Furthermore, there was no significant difference in the total energy intake between the two multiple-food meals (Rolls, B.J. et al., 1983).

Clifton, Burton, and Sharp (1987) presented rats with single-food and multiple-food meals that consisted of four 30-min courses. In the single-food meal each course had the same flavour, whereas in the multiple-food meal, each course consisted of a different flavour (vanilla, peppermint, lemon, and table salt). The flavours were added to wet mash made by soaking lab chow with water. During the last three courses, rats consumed 23% more (grams) of the multiple-food meal than of the single-food meal. Again, this finding supports the conclusion that dietary variety may lead to increased food intake.

The effect of variety on food consumption has also been demonstrated in hamsters (DiBattista & Sitzer, 1994). This species is of particular interest because other factors, such as food deprivation, 5-thioglucose, and 2-deoxy-D-glucose, that tend to increase food intake in other species have been shown not to increase food intake in golden hamsters (DiBattista, 1982; DiBattista, 1984; DiBattista, 1987). Hamsters were provided with four single-food meals and one multiple-food meal consisting of four 12-min courses. In the multiple-food meal, each course consisted of a different food (rodent chow, cheese, cookie, and chocolate), while the four single-food meals had the same food offered during each course. The hamsters consumed 40 - 100%
more energy in the multiple-food meal than in the single-food meals. The effects of dietary variety on food intake were observed only during the last three courses because in the first course of the multiple-food meal dietary variety was not yet available (DiBattista & Sitzer, 1994). Because many factors that have been shown to affect food intake in other species have been shown to have less of an effect on food intake in hamsters, it is noteworthy that dietary variety increased energy intake in golden hamsters. These results not only provide further evidence that dietary variety can influence food consumption, but they also indicate that the effect is not species specific.

Rolls, B.J., Rowe et al. (1981) conducted research to investigate whether dietary variety also affects human food consumption. In their first experiment, participants had access to single-food and multiple-food meals consisting of four 8-min courses. In the multiple-food meal, sandwiches with different fillings (egg, tomato, cheese, and ham) were used with a different type of sandwich being presented in each course. In the single-food meal, the same type of sandwich was presented during each course. Participants consumed approximately 32% more food (grams) in the multiple-food meal than in the single-food meal. In a second experiment, three flavours of yogurt (hazelnut, blackcurrant, and orange) differing in flavour, colour, and texture were used as the test foods. At various times, participants had access to three single-food meals and one multiple-food meal consisting of three 10-min courses. In the multiple-food meal, a different flavour was presented during each course, whereas in the single-food meals, the same flavour was presented in each course. Individuals consumed 20% more (grams) of the multiple-food meal than of the average of the single-food meals. Also, participants consumed 13% more (grams) of the multiple-food meal than of the most preferred single-food meal. In both of these experiments, the
increased intake in the multiple-food meal was observed in the courses following the first course, but not in the first course itself (Rolls B.J., Rowe et al., 1981). These results indicate that dietary variety can enhance food intake in humans.

A Possible Mechanism Mediating the Effects of Dietary Variety: Stimulus-Specific Satiety

The evidence reviewed above indicates that dietary variety can enhance food intake by 13% to 100% in a variety of species. It has been suggested that the stimulatory effect of dietary variety may occur because satiety for foods is at least partly sensory-specific. Sensory-specific satiety occurs when the pleasantness of a food decreases as it is eaten, while the pleasantness of foods that have not been eaten remains relatively unchanged (Rolls, Van Duijvenvoorde, & Rolls, 1984). However, it has been suggested that stimulus-specific satiety maybe a better term than sensory-specific satiety “because it does not presuppose an explanation in terms of a sensory rather than a motivational mechanism” (Clifton et al., 1987, p.149). It is possible that sensory properties may be driving this enhancement of intake, but it may also be to some extent a result of the nutritional properties of the foods that are available. Accordingly, the term stimulus-specific satiety will be utilized throughout this thesis.

Stimulus-specific satiety may form part of the explanation of the intake-enhancing effects of dietary variety. In all meals, the consumption of food may decrease as the meal progresses at least in part because of reductions in the palatability of food that is consumed. In multiple-food meals, the palatability of a food that is eaten will decrease. However, the palatability of other available foods that have different stimulus properties (for example, texture, flavour, nutrients, etc.) will remain high, stimulating further intake. In contrast, in a single-food meal other foods
with different stimulus properties will not be available to stimulate further intake. Consequently, total food intake in multiple-food meals will be higher than in single-food meals (Clifton et al., 1987; Rolls, B.J. et al., 1984).

The role of the extent of variation in stimulus properties in stimulus-specific satiety. Evidence that the effects of dietary variety may be accounted for with references to stimulus-specific satiety is provided by research conducted by Mook, Gonder-Frederick, Keats, and Mangione (1984). On four occasions, rats had access to 2 M glucose solution for 40 min (first course), followed by a second solution for another 40 min (second course). On the four occasions, the second course consisted of (a) the same 2M glucose solution as in the first 40 min, (b) a liquid food that was half sweetened condensed milk and half tap water, (c) the same liquid food as on the second occasion except that it was diluted by half with water, and (d) the same liquid food as on the second occasion of testing. Intake of the liquid milk food, regardless of dilution, was greater than the intake of the 2M glucose solution during the second 40-min course. Specifically, during the second course rats consumed 150-275% and 400% more milliliters of the non-diluted liquid milk and the diluted liquid milk food than the 2M glucose solution, respectively. Therefore, even though rats developed satiety towards one liquid food during the first 40-min course, when a different liquid food was presented during the second course, the rats consumed more of it. Thus, variety in the type of liquid foods available enhanced consumption, lending further support to the idea that dietary variety can lead to increased food intake. Furthermore, in the context of this experiment, satiety was specific to the first liquid food and not to liquid foods in general (Mook et al., 1984).
In the research of Mook et al. (1984), the liquid foods that were used differed in both their sensory and their nutritional properties. Accordingly, it cannot be determined whether enhancement results from differences in their sensory properties (e.g. taste, colour, texture) or in their nutritional properties (e.g. macronutrient composition). However, it has also been demonstrated that introducing the same food substance in a different form can also lead to increased intake (Mook, Brane, & Whitt, 1983). In this experiment, rats were given access to a 2M glucose solution for 40 min (first course) and then access to a second food for another 40 min (second course). On two occasions the second food was the same 2M glucose solution. On other occasions, during the second course rats received either glucose or sucrose in powdered form (4 kcal/g). In the second course, rats derived 267% and 533% more kilocalories from the powdered glucose and sucrose, respectively, than from the 2M glucose solution. In addition, rats consumed 73% more kilocalories of the sucrose powder than the glucose powder. These findings indicate that simply changing the form of a particular nutrient can reinstate feeding, and that the satiety for one form of the nutrient is specific and does not carry over to the second form of the same nutrient. Another finding was that changing both the form and the type of sugar can lead to a greater enhancement of food intake than changing only the form of the sugar (Mook et al., 1983). However, rats show a greater preference for sucrose than for glucose (DiBattista, 1992), and this may be what led to the enhanced intake. Therefore, it appears that greater differences in the sensory properties of the foods available in a multiple-food meal are associated with greater enhancement of food intake. Thus, varying the sensory properties of foods available within a meal may be enough to enhance intake, even without varying their nutritional properties.
Rolls, B.J., Rowe et al. (1981) also conducted research to investigate how the extent of the variety in the properties of foods influences consumption. The studies involved the use of single-food and multiple-food meals which consisted of three consecutive ten-minute courses. In one experiment, yogurts differing in flavour, colour, and texture were presented during each course of the multiple-food meal. In the single-food meal, the same yogurt was presented during each course. Individuals consumed approximately 20% more (grams) in the multiple-food meal than in the single-food meals. In a second experiment, the three yogurts differed in flavour, but they were similar in colour and texture. Under these circumstances, individuals did not consume more food (grams) in the multiple-food meal. Thus, intake increased when the foods in the multiple-food meal differed in several qualities, but not when they differed only in flavour. In this case, it appears that manipulation of only one of the sensory properties of a food may not be sufficient to lead to an increase in food intake.

In the experiments of Rolls, B.J., Rowe et al. (1981), manipulating only the flavour of a food did not enhance intake in multiple-food meals. However, although the foods used in the multiple-food meals differed in flavour, all of the foods used were sweet and thus they were similar in a salient taste property (Rolls, B.J., Rowe et al., 1981). In another experiment, Rolls, B.J., Rowe, and Rolls (1982) manipulated only the flavours of the food, but the foods used were not all sweet. The food used was cream cheese made into sandwiches and flavoured with either curry, table salt, or a mixture of lemon essence and saccharin. Single-food and multiple-food meals were used. The meals consisted of three 7-min courses with the same flavour presented in each course of the single-food meal and a different flavour presented during each course in the
multiple-food meal. Individuals consumed approximately 16% more energy (kJ) in the multiple-food meal than in the single-food meal. Therefore, variety in the flavour of foods may enhance intake if the flavours differ substantially from each other.

It has also been demonstrated that flavour variety can lead to an increase in water consumption. Rats provided with four different flavours of water (orange, raspberry, malt, and peach), one during each of four consecutive 15-min time periods, consumed 53% more water than did rats provided with a single flavour of water (as reviewed by Roll, B.J. & Rolls, 1982). This provides support that simply changing the sensory properties of the ingested substance can affect intake, even if there are no energy benefits derived from increased intake.

It has been demonstrated that manipulating the stimulus property of flavour in some circumstances can increase food intake, but not in others (Rolls, B.J., Rowe et al., 1981; Rolls, B.J. et al., 1982). Manipulating stimulus properties other than flavour may also affect food intake. Rolls, B.J. et al. (1982) examined the effects of manipulation of the colour of food on intake in single-food and multiple-food meals. In the single-food meal, individuals received the same coloured SMARTIE™ (coated chocolate) in each of four 7-min courses, whereas in the multiple-food meal, individuals received a different colour of SMARTIE™ in each successive course. Subjective pleasantness of the taste decreased for the colour that was consumed, but variety in colour did not lead to an increase in food intake. In a second experiment, the same researchers manipulated the shape of the pasta that individuals received. Participants were given either the same shape of pasta in each of three successive 7-min courses (single-food meal), or they were given different shapes during successive courses (multiple-food meal). Individuals' subjective
pleasantness of the taste of the shape they consumed decreased more than that of shapes they did not consume, and furthermore, participants ate about 15% more pasta in the multiple-food meal than in the single-food meal. Therefore, it appears that manipulation of certain aspects of a food's properties can lead to enhanced food intake, while manipulating other aspects may not. In addition, it seems that the greater the manipulation, the greater is the enhancement of the variety effect.

The role of palatability in stimulus-specific satiety. Stimulus-specific satiety appears to be a reasonable explanation of the effects of dietary variety on food intake, as it has been demonstrated that variety involving even one property of a food can lead to increased food intake. Furthermore, there is evidence to indicate that stimulus-specific satiety may depend on reductions in palatability that are to a large extent specific to food that has been consumed. Rolls, B.J., Rolls, Rowe, & Sweeney (1981) found that liking decreases more for a food that has recently been consumed than for other foods that have not been consumed. In their first experiment, individuals rated the pleasantness of the taste of eight foods, consumed one of these foods for a meal, and then at 2 and 20 min afterwards rated the foods again. At both times, ratings decreased more for the food consumed than for the foods not consumed, suggesting that stimulus-specific satiety may be mediated by changes in the hedonic value of ingested foods.

Rolls, B.J., Rolls et al. (1981) also investigated whether the changes in liking would affect food intake. In this second experiment, individuals rated the pleasantness of the tastes of eight foods, consumed one of the foods to satiety, 2 min afterwards re-rated the foods again, and then were given a second course of food that they were not expecting. In the single-food meal
condition, individuals were given either cheese on crackers or sausage in each of two courses, and in the multiple-food meal condition, they were given sausage in one course and cheese on crackers in the other. During each course, the individual was instructed to eat until he/she felt satisfied. Just as in their first experiment, Rolls, B.J., Rolls et al. (1981) found that ratings decreased more for the food consumed than for the foods not consumed, again suggesting that stimulus-specific satiety may be mediated by changes in the hedonic value of ingested foods. Furthermore, individuals consumed approximately 107% more kilocalories in the second course of the multiple-food meal than in the second course of the single-food meals. These findings suggest that the decrease in pleasantness was specific to the food consumed during the first course and that this change in pleasantness decreased food intake in the second course of the meal to a greater extent if the same food was presented, rather than if a different food was presented (Rolls, B.J., Rolls et al., 1981).

Rolls, B.J., Van Duijvenvoorde, and Rolls (1984) have demonstrated that food consumption is greater when the four courses of a meal consist of different foods (multiple-food meal) rather than the same food (single-food meal). In this experiment, individuals consumed 60% more energy (kJ) and 44% more food (grams) in the multiple-food meal than in the single-food meal. The researchers also had the subjects rate the pleasantness of the food. In the single-food meal, the pleasantness of the food declined with time as the food was being consumed during the course of the meal. In the multiple-food meal, consumption of one type of food in a course reduced the pleasantness ratings of that particular food, but did not affect the pleasant rating of foods in later courses that had not yet been consumed (Rolls et al., 1984). The lower intake in the
single-food meal compared to the multiple-food meal may therefore be attributable to stimulus-specific satiety.

Temporal effects in stimulus-specific satiety. Clifton et al. (1987) demonstrated in rats that stimulus-specific satiety to a particular flavour is relatively short-lasting and can be reversed quite quickly. In this experiment, there were two multiple-food meals and one single-food meal, each consisting of three 15-min courses. The foods used in all meal types were nutritionally identical and differed only in sensory properties. The foods were comprised of low-sugar granulated rusk mixed with water, and flavoured with either vanilla, peppermint, or table salt. In the three-food meals, a different flavour of food was presented during each course. In the two-food meal, the same food was presented in the first and third courses and a different food presented in the second course. In the single-food meal, the same food was presented during each course. Compared to intake of the single-food meal, total gram intake was 26% and 21% greater in the two-food and three-food meals, respectively. Furthermore, the final course was approximately 100% and 83% larger in the two-food and three-food meals, respectively, than in the single-food meal. However, there was no significant difference in the amount of food that rats consumed in the final course of the two multiple-food meals. Therefore, presentation of a novel food during the third course did not enhance intake more than presenting a food that had been previously presented in the first course. This suggests that the stimulus-specific satiety that was developed for the food was quickly extinguished by the presentation of a second food, so intake of the original food when it was presented a second time was not affected by its previous presentation.
Effects of stimulus-specific satiety are typically considered to be short-term, but under some circumstances there may be long-lasting effects. The temporal characteristics of stimulus-specific satiety have been studied in the setting of a refugee camp (Rolls, E.T. & De Wall, 1985). In this study, refugees that were in a camp for either two days or six months rated the palatability of three foods that they regularly consumed and three foods that they did not normally consume. Long-term refugees rated the palatability of the regularly consumed foods significantly lower than the three foods not normally consumed, while short-term residents gave all foods similar palatability ratings.

Meiselman, deGraaf, and Lesher (2000) used an experimental paradigm to study the effects on food intake of long-term dietary variety. They used two treatment conditions, a variety condition and a monotony condition. Dietary variety was available within the meals of both conditions, but individuals in the monotony condition consumed the same meal each day over a five-day period. Individuals in the variety condition consumed the same meal on days one and five, but consumed different meals on the three intervening days. Therefore, comparing the intake on days one and five in the two treatment conditions allows for the determination of the effects of eating a variety of meals over a number of days compared to eating the same meal over a number of days. In the monotony condition, intake of each of the foods within the meal tended to decline from day one to five, whereas in the variety condition intake of each of the foods within the meal tended to be greater on day five than on day one (Meiselman et al., 2000). These results indicate that dietary monotony over a number of days can suppress food intake, while dietary variety may enhance intake.
The increased intake associated with dietary variety may be implicated in the development of obesity. Rolls, B.J. et al. (1983) found that rats that had simultaneous access to two foods consumed at least 15% more energy during a 36-hr period than rats that had access to a single food. Furthermore, rats that had continuous access to two foods over a seven-week period gained on average 12% more weight and had 22% greater fat deposits than the rats that had access to only one food (Rolls, B.J. et al., 1983).

**Summary and Conclusions**

In summary, the research presented suggests that stimulus-specific satiety may mediate the intake-enhancing properties of dietary variety. Furthermore, stimulus-specific satiety is, for the most part, a short-term phenomenon, although it has some long-term effects as well. In addition, it has also been found that the effects of dietary variety on food intake increases as a function of the magnitude of the differences in the properties of the foods available in a multiple-food meal. Moreover, stimulus-specific satiety may be a result of reductions in palatability that tend to be specific to foods that have been previously consumed. Thus, in a single-food meal, continued exposure to a particular food will lead to a decrease in palatability which will contribute to the decline in intake that occurs over time. In contrast, in a multiple-food meal, the consumption of a particular food will have little effect on the palatability of the other foods that are available within the meal. As a consequence, total food intake will tend to be greater in multiple-food meals than in single-food meals.
The Role of CCK in the Control of Eating Behaviour

The CCK pathway is one of a number of gastrointestinal processes involved in the control of food intake. CCK, a gut peptide, is released during the ingestion of food from the upper small intestine (Smith & Gibbs, 1998). The role of CCK in satiety has been studied extensively, mainly through the use of the C-terminal octapeptide of CCK (CCK-8). Both the specifics of how CCK exerts its effects and the nature of its role in satiety continue to be debated.

The Release of Endogenous CCK

In very early studies, it was demonstrated that endogenous CCK is released after consumption of food or when food is placed in the small intestine. Wang and Grossman (1951) examined the effects of the placement of various solutions into the small intestine of dogs on secretion from a transplanted pancreas. In previous research, it has been demonstrated that injection of pancreozymin extracts, now known to be the same peptide as CCK, stimulated pancreatic enzyme output, but had no effect on the volume of the secretion (as reviewed by Wang and Grossman, 1951). Therefore, in their experiment Wang and Grossman (1951) used an increase in enzyme output per minute as a measure of the release of pancreozymin (i.e. endogenous CCK). The placement of an amino acid solution in the small intestine caused a 206% and a 76% increase in the rate of amylase output during the first and second 20 min periods of secretion collection, respectively. During the third 20 min period, there was no increase in amylase output. The introduction of a corn oil solution into the small intestine led to a 11%, 57%, and 30% increase in amylase output during the first, second, and third 20 min time periods, respectively. The introduction of three different carbohydrate solutions into the small intestine did
not lead to a significant increase in amylase output during any of the 20 min time periods (Wang & Grossman, 1951). These results suggest that the entrance of certain nutrients into the small intestine causes a release of endogenous CCK.

Harvey, Dowsett, Hartog, and Read (1973) demonstrated that endogenous CCK is released when nutrients are consumed. They studied this phenomenon through the use of a specific radioimmunoassay which allows for the measurement of physiological levels of CCK in human blood taken from the periphery. The ingestion of milk, amino acids, olive oil, and magnesium sulphate led to a rapid increase in serum CCK levels. The levels peaked between 15 and 45 min following ingestion, with levels returning near base line between 45 and 60 min (Harvey et al., 1973). These results provide support that endogenous CCK is released after the consumption of food. Furthermore, the results of both Wang et al. (1951) and Harvey et al. (1973) suggest that the effects of endogenous CCK may be relatively short lasting.

Effects of Exogenous CCK on Food Intake

The inhibitory effect of exogenous CCK on food intake has been demonstrated repeatedly. In an early study, Gibbs et al. (1973) discovered that partially purified CCK suppressed intake of both liquid and solid foods in a dose-dependent manner. Five-and-a-half-hr food-deprived rats were injected intraperitoneally (i.p.) with partially purified CCK or 15M saline 15 min prior to food presentation. Rat chow was available for 150 min and intake was measured every 30 min. Partially purified CCK administered in doses of 5, 10, 20, and 40 Ivy Dog U/kg inhibited solid food intake by 25%, 35%, 35%, and 45%, respectively, during the first 30 min of food availability. Gibbs et al. (1973) conducted another experiment examining the effects of CCK
on liquid food intake in 17-hr food-deprived rats, with intake being measured at 5, 15, 30, 60, 90, 120, and 150 min. CCK inhibited intake most markedly during the 5-15 min interval. The 10 Ivy Dog U/kg dose of partially purified CCK caused reductions in intake of 7% from 0 to 5 min, and 31% from 5 to 15 min; however, there were no further reductions in intake either from 15 to 30 min, or from 30 to 60 min (Gibbs et al., 1973). In summary, Gibbs et al. (1973) found that partially purified CCK inhibited intake of both solid and liquid foods in a dose-dependent manner and that the effects of exogenous CCK are fairly short lasting.

Manipulating the timing of the injection of exogenous CCK prior to the presentation of food provides further evidence that the effects of CCK are short lasting. For example, Kulkosky, Sanchez, Foderaro, and Chiu (1989) injected 23-hr water-deprived rats with saline or one of four doses of CCK-8 (0.25, 0.50, 1.00, or 2.00 μg/kg) either 0, 10, or 20 min prior to the presentation of a 5% ethanol solution for 30 min. The 0.25 and 0.50 μg/kg doses of CCK did not reduce intake regardless of the time of administration. The 1 and 2 μg/kg doses of CCK significantly reduced ethanol intake by approximately 28% and 22%, respectively, when administered 10 min prior to ethanol presentation and by 30% and 40%, respectively, when administered 0 min prior to ethanol presentation. However, the 1 and 2 μg/kg doses of CCK did not significantly reduce intake when administered 20 min prior to the presentation of ethanol (Kulkosky et al., 1989). Thus, the effects of exogenous CCK are short lasting.

Other researchers have also confirmed the finding of Gibbs et al. (1973) that exogenous CCK reduces food intake even in food-deprived rats. For example, Moran et al. (1997) demonstrated that 5-hr food-deprived rats ate less when injected i.p. with CCK prior to eating.
Rats were injected with saline or one of five doses of CCK-8 5 min prior to access to a glucose solution for 30 min. The 1, 2, 4, 8, and 16 μg/kg doses of CCK inhibited food intake by 17%, 34%, 54%, 54%, and 70%, respectively. In another study, using the same procedures, Moran et al. (1998) demonstrated that 1, 2, 4, 8, and 16 μg/kg doses of CCK reduced food intake in 5-hr food-deprived rats by 29%, 43%, 62%, 67%, and 71%, respectively. These findings clearly indicate that exogenous CCK can cause animals to reduce their food consumption in the short term and that the effect of CCK is dose-dependent.

Exogenous CCK has also been shown to inhibit sham feeding as well as real feeding. For example, Le Sauter et al. (1988) demonstrated that CCK-8 injected i.p. into either non-deprived or 18-hr deprived rats inhibited sham feeding. In this study, a gastric cannula was surgically implanted into each rat, and during the feeding test the cannula was left open so that ingested food did not reach the small intestine. In non-deprived rats, a 1.0 μg/kg dose of CCK inhibited sham feeding of milk by approximately 90%. In food-deprived rats, 1.0 and 2.0 μg/kg doses of CCK inhibited sham feeding of milk by approximately 50% and 40%, respectively. These results suggest that neither the presence of nutrients in the small intestine, nor the postabsorptive events that normally follow ingestion are necessary for exogenous CCK to inhibit intake.

It appears that CCK is present not only in the periphery, but also in the central nervous system (CNS), and that central CCK also plays a role in the control of feeding behaviour (Schick, Yaksh, & Go, 1985). Zhang, Bula, and Stellar (1985) demonstrated that injection of CCK-8 into the anterior cerebral ventricles of the rat decreased both eating and running speed toward food rewards. A 2.0 μl/kg dose of CCK inhibited intake of wet mash by approximately 75% and 51%
at 15 and 30 min into the 60-min feeding test, respectively. Studies have also been performed to
determine whether centrally administered CCK may in fact be acting peripherally as a result of its
redistribution to the periphery via the bloodstream. For example, Schick, Schusdziarra, Yaksh,
and Go (1994) injected CCK-8 into 24-hr fasted rats via either an intracerebroventricular or an
intravenous route. Intracerebroventricular injection of CCK-8 delayed the onset of eating and
reduced the amount consumed by 45%, although the plasma levels of CCK were not altered by
this injection. Intravenous injection of CCK-8 did not suppress eating behavior in fasted rats until
injected at doses high enough to increase the plasma level to ten times the base level. Thus,
centrally administered CCK appeared to be acting centrally rather than peripherally, supporting
the possibility of central control of eating behavior by CCK (Schick et al., 1994).

CCK and Malaise

In establishing a role for CCK as a satiety factor, it is important to determine whether
CCK actually acts as a satiety signal or whether the reduction in food intake caused by CCK is
merely the result of malaise. A number of studies have been conducted to determine whether CCK
causes some form of malaise and thereby decreases food consumption. Studies have involved
conditioned taste aversion, conditioned discrimination, taste reactivity, human self-reports, and
other methodology, with the majority supporting the conclusion that CCK does not have a toxic
effect and that CCK probably acts as a satiety signal under normal circumstances (as reviewed by
Smith & Gibbs, 1998).

In an early study, Antin, Gibbs, Holt, Young, and Smith (1975) examined whether CCK
administration elicits the normal behavioural sequence associated with satiety in rats. In this
research, they first demonstrated that there is a marked difference in the behaviours of real-feeding and sham-feeding rats. They found that when rats consume a liquid diet, they display a behavioural sequence that involves a slowing down in the rate of eating behaviour, and at the same time there is an increase in the frequency of occurrence of other behaviours, including sniffing, grooming, and locomotion. After several minutes, these behaviours cease and resting occurs. In contrast, Antin et al. (1975) found that sham feeding did not elicit this sequence of behaviours. In rats that were sham fed, the ingestion of a liquid diet did not lead to a slowing in the rate of eating behaviour. These rats fed for approximately 78% of the time of a 60-min test period and on the occasions they did stop, they stopped only briefly. On the other hand, rats with a closed gastric fistula fed for only 15% of the test period. Sham feeding rats did exhibit sniffing, grooming, and locomotion, but they did not exhibit any resting after these behaviours ceased. Therefore, an examination of the sequence of behaviours that occurs when feeding ceases can be used to determine whether the termination of an animals’s eating behaviour represents normal satiety. For example, because quinine is one substance that has been shown to slow or stop feeding, Antin et al. (1975) presented a quinine sulfate solution 12 min into sham feeding of a liquid diet to determine whether this solution would elicit the normal behavioural satiety sequence. Upon presentation of the quinine solution, feeding stopped almost immediately, and the rats became active and did not rest. Therefore, quinine does not elicit the satiety sequence. Finally, the effects of CCK on feeding and behaviour were examined by injecting rats with a 40 Ivy Dog U/kg dose of CCK after 12 min of sham feeding of a liquid diet. After administration of CCK, feeding slowed and stopped in all animals by 36 min, with CCK inhibiting food intake by 55%. As feeding
decreased, rats displayed sniffing, grooming, and locomotion, and then they rested (Antin et al., 1975); that is, they displayed the normal behavioural sequence associated with satiety. These findings strongly suggest that exogenous CCK elicits the complete behavioural sequence of satiety, and that feeding is not discontinued due to malaise when CCK is administered.

West, Greenwood, and Marshall (1987) also studied whether the effects of CCK were due to malaise by comparing the effects of long-term infusion of CCK and lithium chloride (LiCl) on food intake in rats. LiCl is a nausea-inducing agent that is frequently used in studying conditioned taste aversions. Infusion of CCK significantly shortened meals and reduced meal size, but meal frequency increased. In contrast, infusion of LiCl led to a reduction in the frequency of feeding, but it did not affect either meal duration or size. These findings suggest that infusion of LiCl caused an aversion to feeding, while CCK did not. Thus, it appears that infusion of CCK at doses that effectively reduce meal size does not cause malaise in rats (West et al., 1987).

**Effects of CCK Antagonists on Food Intake**

Two subtypes of cholecystokinin receptors have been identified: CCK$_A$ and CCK$_B$. Both forms of receptors have been located in both the periphery and the central nervous system, with CCK$_A$ receptors predominating in the periphery and CCK$_B$ receptors predominating in the brain (Corp, Curcio, Gibbs, & Smith, 1997). CCK antagonist studies have provided evidence supporting a role for CCK in the control of feeding.

Studies in which CCK antagonists are administered in conjunction with exogenous CCK provide clear evidence that CCK interacts with CCK receptors to reduce food intake. In one study, rats injected with CCK-8 (8 nmol/kg dose) reduced their food intake by 48%. However,
when rats were injected with either a 0.1 or a 0.3 mg/kg dose of the CCK$_A$-antagonist L 364718 (devazepide) 25 min prior to injection with CCK-8, the effects of CCK on food intake were completely reversed (Reidelberger & O'Rourke, 1989). Silver, Flood, Song, and Morley (1989) performed a similar study in which they administered both CCK-8 (10 $\mu$g/kg dose) and the CCK$_A$-antagonist devazepide (1.0 mg/kg dose) to mice. The CCK-induced decrease in food intake was completely reversed by the devazepide (Silver et al., 1989). These results suggest that exogenous CCK inhibits food intake by binding to CCK$_A$ receptors, and that its actions are not merely the result of nonspecific effects.

Studies with other CCK$_A$ antagonists have provided results that are consistent with those in which devazepide has been used. For example, when 0.2 and 2 mg/kg doses of the CCK$_A$-antagonist lorglumide are administered i.p. to rats, the inhibitory effects of an i.p. injection of an 8 $\mu$g/kg dose of CCK-8 are antagonized by 62% and 82%, respectively (Schneider, Murphy, & Smith, 1988). It has also been demonstrated that intravenous infusion of lorglumide antagonizes the inhibitory effects of centrally administered CCK-8 on food intake in dogs (Inui, Inoue, Sakatani, Oya, Morioka, & Baba, 1987). Furthermore, lorglumide appears to act through peripheral mechanisms rather than central ones, as injection of lorglumide into the lateral ventricle does not reverse the inhibitory effects of peripherally administered CCK (Corp et al., 1997).

Experiments in which CCK$_A$ antagonists are administered alone (i.e., without exogenous CCK also being administered) provide important information about the role of endogenous CCK in the control of eating behaviour. Utilizing CCK$_A$ antagonists allows for examination of the effects of endogenous CCK on food intake by blocking or partially blocking the effects of
endogenous CCK. If endogenous CCK is involved in the normal termination of feeding, then blocking the effects of endogenous CCK should lead to an increase in food intake. In one such study, rats injected with 0.1, 0.3, and 1.0 mg/kg doses of the CCK$_A$-antagonist devazepide 30 min prior to the presentation of ground chow increased their intake by 11%, 20%, and 23%, respectively, during the 3-hr period after the initial food presentation (Reidelberger et al., 1989). In another study, injection of a 750 μg/kg dose of devazepide into lean male Zucker rats 60 min prior to access to liquid food for 60 min increased their food intake by 27% or more (Strohmayer & Greenberg, 1996). Silver et al. (1989) found a similar effect of devazepide on food intake in mice. Non-fasted mice injected i.p. with a 100 μg/kg dose of devazepide 30-min prior to the presentation of rodent chow increased their food consumption by 45%. However, devazepide did not increase intake in 18-hr fasted mice (Silver et al., 1989). These findings suggest a role for endogenous CCK in the reduction of food intake.

Mechanisms and Site of Action of CCK

A number of studies have determined that CCK decreases food intake during a meal, has short-lasting effects, and may be involved in signaling meal termination (Gibbs et al., 1973; Le Sauter et al., 1988; Moran et al., 1998; West et al., 1984). The mechanism and the location of the sites involved in regulation of food intake by CCK have also been examined. CCK is distributed throughout the gastrointestinal tract, but tends to be concentrated in the jejunum and the duodenum (as reviewed by Baldwin, Parrott, & Ebenezer, 1998). CCK has been shown to activate gallbladder contractions, stimulate pancreatic enzyme secretion (Silver & Morley, 1991), affect intestinal motility, and inhibit gastric emptying (Moran, 2000). Some of these functions of
CCK appear to be paracrine or neurocrine in nature, whereas others appear to be endocrine in nature. CCK is also located in the brain in variable concentrations and has met some of the criteria for being a neurotransmitter (Moran, 2000). There is evidence that CCK activates CCK\textsubscript{A} receptors in the gastrointestinal tract and that signals from this stimulation is sent to the nucleus tractus solitarius via the vagus nerve. From the nucleus tractus solitarius the signal is transmitted to higher centers, including the lateral parabrachial nucleus and central nucleus of the amygdala (Bray, 2000; Schick et al., 1994).

**CCK Receptor Types.** The importance of CCK receptors in the mediation of CCK’s suppressive effects on food intake has been studied. As previously mentioned two subtypes of CCK receptors have been identified: CCK\textsubscript{A} and CCK\textsubscript{B}. Both forms of receptors have been located in both the periphery and the central nervous system, with CCK\textsubscript{A} receptors predominating in the periphery and CCK\textsubscript{B} receptors predominating in the brain. CCK\textsubscript{A} receptors have been located on the gastrointestinal tract, the vagus nerve, and the pancreas (Corp et al., 1997). The CCK binding sites in the gastrointestinal tract are located in the gastroduodenal region and are found mainly in the circular muscle of the pyloric sphincter. It is believed that CCK causes contraction of the pyloric sphincter by acting directly on CCK receptor binding sites (Smith, Falasco, Moran, Joyner, & Gibbs, 1988). The binding sites on the pyloric sphincter may also play a role in the decrease in food intake that is caused by CCK (Conover, Collins, & Weingarten 1989; Moran & McHugh, 1982; Shillabeer & Davison, 1987). CCK\textsubscript{A} receptors have also been either detected or inferred in the dorsal medial nucleus of the hypothalamus, the nucleus accumbens, and the nucleus of the
solitary tract (Corp et al., 1997). CCK<sub>B</sub> receptors have been located on the vagus nerve and in other peripheral tissues, as well in the CNS.

The CCK<sub>A</sub> receptors, but not the CCK<sub>B</sub> receptors, appear to be the primary mediators of the satiating effects of peripherally administered CCK. In one experiment, 5-hr food-deprived rats were injected i.p. 30 min prior to food presentation either with vehicles, or with a range of doses of either the CCK<sub>A</sub>-antagonist devazepide or of the CCK<sub>B</sub>-antagonist L365260. Then the rats were injected i.p. with a 4 µg/kg dose of CCK-8 5 min prior to having access to a glucose solution for 60 min. Devazepide antagonized the inhibitory effects of CCK in a dose-dependent manner, whereas L365260 did not antagonize the effects of CCK. This result suggests that the effects of peripherally administered CCK are mediated by CCK<sub>A</sub> receptors (Moran, Ameglio, Schwartz, & McHugh, 1992). The effects of these two antagonists on endogenous CCK have also been examined. The procedures were similar to those of the previous experiment except that the rats did not receive the injection of exogenous CCK. Administration of 32 and 100 µg/kg doses of devazepide increased intake of glucose solution by 39% and 44%, respectively, but administration of a range of doses of L365260 did not affect intake. These findings suggest that the effects of both exogenous and endogenous CCK may be mediated by CCK<sub>A</sub> rather than by CCK<sub>B</sub> receptors (Moran et al., 1992).

In certain circumstances, an insensitivity to the effects of CCK may play a role in overeating and obesity. The Otsuka Long-Evans Tokushima Fatty (OLETF) rats lack CCK<sub>A</sub> receptors, which have been implicated in the mediation of CCK’s inhibitory effects. Compared to Long-Evans Tokushima rats (LETO) that do not lack CCK<sub>A</sub> receptors, OLETF rats are obese,
exhibit increased meal sizes, a licking rate that declines more slowly, and greater food consumption. Furthermore, OLETF rats are resistant to the inhibitory control of CCK over food consumption. For example, OLETF rats injected i.p. with a range of doses (1, 2, 4, 8, & 16 µg/kg) of exogenous CCK-8 5 min prior to the presentation of glucose solution for 30-min do not decrease their intake. The lack of CCK_A receptors may be related to the hyperphagia and obesity seen in the OLETF rats, suggesting that CCK may play a role in body weight regulation (Moran et al., 1998)

The Role of the Vagus Nerve. Vagal afferent and efferent connections appear to be an important part of the CCK pathway. For example, Le Sauter et al. (1988) examined the effects of CCK on real and sham feeding in rats that had undergone a total abdominal vagotomy, which involved the removal of segments of the vagal branches rostral to the hepatic and celiac branches. In their research they found that sham-operated rats injected i.p with a range of doses CCK-8 reduced their food intake by up to 90%. However, vagotomized rats injected i.p. with the same range of doses of CCK-8 did not reduce their food intake, except when they were injected with 6 µg/kg of CCK (i.e., a large dose). In vagotomized rats, the 6 µg/kg dose of CCK inhibited food intake by 35%, but this dose was much more effective in reducing intake in sham-operated rats, as this dose inhibited intake by 90% in sham-operated rats. The research of Moran et al. (1997) also supports the conclusion that a total abdominal vagotomy removes the inhibitory effects of CCK. These researchers found that total abdominal vagotomy resulted in loss of the suppressive action of CCK on food consumption, whereas sham-operated rats injected i.p. with CCK-8 reduced their
intake in a dose-dependent manner. These findings suggest that abdominal vagal fibres are involved in the mediation of the suppressive effect of CCK (Moran et al., 1997).

**Other Factors that Can Influence the Satiety Effects of CCK**

The inhibitory effects of CCK on food intake interact with certain other factors that may either enhance or diminish its inhibitory effects. One factor that has been shown to enhance the inhibitory effects of CCK on food intake is estrogen. Implantation of estradiol into the paraventricular nucleus increases the inhibitory effects of CCK on food intake in female rats (Butera, Xiong, Davis, & Platania, 1996). In addition, CCK suppresses food intake to a greater extent in ovariectomized rats implanted with an estradiol capsule than it does in ovariectomized rats implanted with an empty capsule (Butera, Bradway, & Cataldo, 1993).

In contrast, the effects of learning and tolerance may inhibit the suppressive effects of CCK (Goodison & Siegel, 1995). In their research, Goodison and Siegel (1995) injected two groups of rats (contingent and saline groups) every second day over a 18 day period with CCK-8 and saline, respectively. Fifteen min after injection, rats were given access to a sucrose diet for 30 min. In addition, a third group of rats (noncontingent group) was injected with CCK at the same time as the other two groups of rats were injected, but this group did not receive the sucrose solution until the following day. During the initial days of testing, the contingent rats ate less than saline rats. However, by the end of the eight days of testing, the saline rats and the contingent rats were consuming the same amount of sucrose during the 30-min period. Thus, the contingent rats demonstrated tolerance to the intake-inhibiting effects of CCK. Furthermore, the noncontingent rats consumed an amount of sucrose solution that was similar to that of the saline rats. At this
point in the study, all three groups of rats were tested twice. On one day the three groups were injected with CCK prior to sucrose presentation, while on the second day they were injected with saline prior to food presentation. The contingent rats, that had developed the tolerance to CCK, consumed more food than either of the other two groups of rats both when injected with saline and with CCK. This suggests that the contingent rats learned to tolerate the effect of CCK after a number of days of testing by associating the administration of CCK with the suppressive effects of CCK. The contingent rats compensated for the effects of the CCK injection by overeating when they were exposed to the cues associated with the effects of CCK, even if saline was the substance injected. Moreover, it was demonstrated that this tolerance did not develop if the administration of CCK was not paired with the suppressive effects associated with CCK.

To further demonstrate that learning plays a role in the development of tolerance, Goodison and Siegel (1995) examined at the effects of environmental contingencies in the tolerance developed by rats towards CCK. In their research, during the tolerance development phase of the experiment, one group of rats (CCK group) were injected with CCK and a second group (saline group) with saline 15 min prior to the presentation of sucrose solution over a number of days. Furthermore, half of the CCK group (CCK-colony group) and half of the saline group (saline-colony group) were tested in the colony room, whereas the other halves of the CCK group (CCK-room group) and saline group (saline-room group) were transferred to a separate testing room. By the end of the tolerance development phase the CCK-colony and the CCK-room rats were consuming similar quantities of food compared to saline-colony and saline-room rats. Next, all groups of rats were tested under three conditions, which included 1) being injected with
CCK prior to sucrose presentation in the colony room, 2) being injected with CCK prior to food presentation in the separate testing room, and 3) being injected with saline prior to food presentation in whatever room that they had been injected in during the tolerance development phase. When rats were injected with CCK in the colony room and in the separate testing room, CCK reduced the intake in all of the groups of rats, except for the rats that had originally developed tolerance to CCK in the particular room. Thus, rats did not display their previous learned tolerance to the suppressive effects of CCK when the CCK injections were administered in the absence of the environmental cues previously associated with the injection. Furthermore, when rats were injected with saline in the rooms that they had been tested in during the tolerance development phase, the saline-colony and saline-room rats consumed food in quantities similar to their intake during that tolerance development phase. However, the CCK-colony and CCK-room rats, that had learned the tolerance to CCK, increased their food intake compared to the amount they were consuming by the end of the tolerance development phase. Therefore, even when they were injected with saline, rats that had developed a tolerance towards CCK compensated for the effects of CCK, because the environmental cues associated with the suppressive effects were still present (Goodison & Siegel, 1995). This demonstrates that learning can have an impact on the effectiveness of CCK’s capacity to reduce food intake.

Effects of CCK on Palatability

It has been proposed that the inhibitory effects of CCK may be mediated by a reduction in the palatability of the ingested substance. Waldbillig and O’Callaghan (1980) suggested that CCK inhibits food intake by affecting the ‘behavior-controlling characteristics of taste’. They examined
the ‘taste hypothesis’ by studying the effects of the presence or absence of sucrose in water on the inhibitory effects of CCK. Waldbillig and O’Callaghan (1980) reasoned that if CCK inhibits the intake of water containing sucrose, but not the intake of water lacking sucrose, then the effects of CCK may be attributable to the taste properties of sucrose. In this study, 15-hr water-deprived rats were injected with either 0.9% saline or a 40 Ivy Dog U/kg dose of CCK-8 and then given access to either tap water or a 0.3M sucrose solution for 15 min. CCK did not inhibit water intake, but it did inhibit the intake of the sucrose solution by 26%, suggesting that the intake-reducing effects of CCK may be mediated by the taste properties of the ingested substance.

Although the reduction in the palatability of the ingested substance is a possible explanation of the intake reducing abilities of CCK, it is also possible that the caloric value of the sucrose solution may be mediating the effects of CCK. Accordingly, Waldbillig and O’Callaghan (1980) tried to determine whether the effects of CCK are mediated by the taste properties of a food or the caloric value associated with the food. They argued that if the taste properties mediate the effects of CCK, then CCK should have no effect on the latency to drink, and it should suppress feeding within the first few minutes after the onset of food presentation. On the other hand, if the caloric value associated with the food mediates the effects of CCK, then suppression should not occur until later in the meal because there must be enough time for the animal to experience the post-ingestive consequences of the food. In this study, rats were injected i.p with either saline or a 40 Ivy Dog U/kg dose of CCK 15 min prior to the presentation of a 0.3M sucrose solution. CCK inhibited intake early in the 15-min feeding test, with intake being significantly suppressed even during the first minute. It was also found that CCK did not affect the
latency to begin drinking. These results suggest that the intake-reducing effects of CCK may be mediated by the taste properties of the 0.3M sucrose solution because it is unlikely that the animal would have experienced the caloric properties of the solution after only one min of intake.

Further research on the taste hypothesis was carried out by Waldbillig and Bartness (1982). They studied the effects of varying concentrations of sucrose solutions on the intake-reducing effects of CCK. They argued that if the effects of CCK are mediated by the orosensory properties of food, then the higher the concentration of the sucrose solution (i.e., the more intense the orosensory properties) the more effective CCK will be at inhibiting intake. In this study, rats were injected with either saline or CCK-8 (40 and 60 Ivy dog U/kg doses) 15 min prior to the presentation of one of either a 0.2%, 10%, or a 35% sucrose solution for 30 min. Intake was measured at 1, 2, 3, 15, and 30 min. In general, CCK's inhibitory effectiveness increased as the concentration of the sucrose solution increased, although there was no difference in CCK's effectiveness at the two higher concentrations. Waldbillig and Bartness (1982) suggested that rats may not have been able to detect differences between these two concentrations of sucrose solutions. These findings provide some support that the effect of CCK may be mediated by the orosensory properties of foods, although caloric density may also play a role if sweetness is associated with caloric density (Waldbillig & Bartness, 1982). The effects of caloric density and sweetness need to be separated to determine the individual effects of each on the suppressive effects of CCK.

Eckel and Ossenkopp (1994) more directly studied whether CCK reduces the palatability of foods. To accomplish this, they made use of the fact that rats exhibit different facial expressions
in response to the presence of palatable versus unpalatable foods in their mouths. When palatable foods are present in the mouth, rats display small amplitude, bilateral symmetric lowering of the mandible (i.e., mouth movements), midline extensions of the tongue over the upper lip (tongue protrusions), and large amplitude, lateral extensions of the tongue resulting in unilateral retraction of the upper lip (i.e., lateral tongue protrusions). On the other hand, when aversive or unpalatable foods are present in the mouth, rats display gaping, head shakes with fluid expulsion, and flailing of the forelimbs (as reviewed by Eckel & Ossenkopp, 1994). In this study, rats were implanted with intraoral cannulas and tubing threaded into the mouth. A 0.30M sucrose solution was infused intraorally for 30 seconds at 2, 4, 6, 8, and 10 min after i.p. injection of saline or one of three doses of CCK-8 (4, 8, or 16 μg/kg). Infusions into the mouth were delivered at a constant rate and the orofacial responses to the infusion was videotaped. In a dose-dependent manner, CCK decreased the ingestive responses to the 0.30M sucrose solution, but it did not increase orofacial responses associated with the administration of aversive stimuli. These findings suggest that CCK reduces the palatability of the 0.30M sucrose solution, and this suggests that when CCK is administered and reduces food consumption that CCK is not doing this by causing malaise. The results of Eckel and Ossenkopp’s (1994) study and the results of the other studies examining the effects of CCK on palatability suggest that the effects of CCK may be mediated, at least in part, by a reduction in the palatability of the ingested substance.

**Summary**

There is strong evidence that CCK is involved in the control of food intake. Peripherally administered CCK has been shown to inhibit food intake in a dose-dependent manner over a
relatively short period of time. This effect is very robust, being observed in deprived and non-deprived animals, in sham-feeding and real-feeding animals, and in the context of solid and liquid foods. Furthermore, CCK has its effects without causing malaise, and animals can learn a tolerance towards the suppressive effects of exogenous CCK, limiting its intake-reducing effects. Vagal innervations, and stimulation of CCK-A and B receptors in both the periphery and the CNS appear to underlie the inhibitory effects of CCK. For example, it has been demonstrated that CCK\textsubscript{A}-antagonists, such as devazepide, can reverse the inhibitory effects of peripherally administered CCK, and may actually increase food intake when administered in the absence of exogenous CCK, suggesting that endogenous CCK may be involved in meal termination under normal circumstances. Finally, it has been suggested that the reduction in food intake that is caused by CCK may be mediated, at least in part, by a reduction in the palatability of the ingested substance.

**Effects of Exogenous CCK on Food Intake in Single-food and Multiple-food Meals**

The preceding review of the literature on dietary variety and on CCK reveals that up to the present time there have been few studies of the effects of CCK administration in the context of dietary variety. That is, with very few exceptions research has focused on the effects of CCK administration when a single food, either solid or liquid, is available for consumption. In one study of the effects of CCK on diet selection, Thibault, Nagai, Hashida, Yanaihara, & Nakagawa (1990) allowed rats to select among separate carbohydrate, fat, and protein sources, and over an eight-day period they infused either a vehicle or the CCK-8 derivative pyroglutamyl-CCK-8 (pGlu-CCK-8) into the ventromedial hypothalamus. They found that pGlu-CCK-8 specifically
suppressed carbohydrate intake, and to a lesser extent fat intake. However, it must be noted that in this experiment there was no treatment group that received a single food source, making it impossible to compare the effects of chronic pGlu-CCK-8 administration in single-food and multiple-food contexts.

Of course in an animal’s natural environment, it will frequently encounter dietary variety when it happens to come upon two or more foods either simultaneously or in rapid succession. Considered from this perspective, research on the effects of CCK administration has generally been less ecologically valid than it might be in that the focus of CCK research has been almost exclusively on situations in which single foods have been available for consumption. One of the goals of this thesis is to address this gap in our knowledge by comparing the effect of exogenous CCK in the context of single-food and multiple-food meals.

A second goal of this thesis is to examine the role that palatability reduction may play in mediating the effects of CCK on food intake. As mentioned above (see The Effects of Palatability on CCK), evidence exists indicating that the intake-reducing effects of CCK are mediated, at least in part, by a reduction in palatability. There are at least two possible ways in which changes in palatability might lead to a reduction in food intake. First, CCK might exert its effects on food intake by reducing the palatability of all foods. According to this all-foods hypothesis, exogenous CCK would reduce the palatability of all foods to a similar degree regardless of whether these foods are actually available to the animal, and if they are available, regardless of whether the animal has recently consumed them. Alternatively, exogenous CCK might exert its effects on food intake by reducing the palatability only of recently consumed foods. According to this recent-
foods hypothesis, CCK would reduce the palatability only of those foods that have recently been ingested and would not affect the palatability of uneaten foods, even if these foods should happen to be present in the animal's immediate environment.

When only a single food is available during a meal both the all-foods hypothesis and the recent-foods hypothesis predict that CCK will reduce the palatability of that food, with the result that food intake will be suppressed. Of course, this CCK-induced reduction in the size of a single-food meal has been observed in numerous experiments. Thus, the administration of CCK in the context of a single-food meal does not permit an evaluation of the relative merits of the all-foods and the recent-foods hypotheses. However, the all-foods and the recent-foods hypotheses lead to different predictions when considered in the context of multiple-food meals. If the all-foods hypothesis is correct, CCK would have the same suppressive effect on the palatability of all foods that an animal may happen to encounter during a meal, and it should therefore have the same suppressive effects on total food intake regardless of the number and the nature of the foods available during a meal. In summary, if the all-foods hypothesis is correct the intake reducing effects of exogenous CCK should be similar in single-food and in multiple-food meals.

Accordingly, in an experiment in which both meal type (single-food and multiple-food) and CCK dose are independent variables and total food intake is the dependent variable, the all-foods hypothesis predicts that there should be no interaction between the two independent variables. Specifically, the intake-reducing effect of exogenous CCK should be similar in the single-food and the multiple-food meal conditions.

On the other hand, if the recent-foods hypothesis is correct, exogenous CCK should be
much less effective in reducing food intake in multiple-food than in single-food meals. Of course, in a single-food meal, CCK would cause a reduction in the palatability of the food as it is consumed with the result that meal size would be substantially lower in CCK-injected than in vehicle-injected animals. In addition, in a meal in which several distinctly different foods are available, CCK would cause a reduction in the palatability of the first ingested food as it is consumed. However, when the animal stops eating the first food it would start eating the other available foods, and because these foods would have retained their initial levels of palatability, intake of these foods would be substantially higher than it would be if their palatability had already been reduced by CCK administration (as would be predicted by the all-foods hypothesis).

Accordingly, in an experiment in which both meal type (single-food and multiple-food meals) and CCK dose are independent variables and total food intake is the dependent variable, the recent-foods hypothesis predicts there should be a significant interaction between the two independent variables. Specifically, the intake-reducing effect of exogenous CCK should be significantly greater in the single-food meal condition than in the multiple-food meal condition.

In the experiments to be reported here, the intake-reducing effect of exogenous CCK will be examined in the context of single-food and multiple-food meals. In all cases, multiple-food meals will consist of three foods that differ substantially from each other in their sensory properties and CCK will be administered by the intraperitoneal route just before food is presented for a 30-min period. In Experiment 1 through 3, various doses of CCK will be administered and the foods within the multiple-food meals will be presented simultaneously. In Experiment 4, the foods in the multiple-food meal condition will be administered consecutively rather than
simultaneously. Finally, Experiments 5 and 6 will be conducted to examine the effects of the CCK\textsubscript{A}-receptor-antagonist lorglumide in the context of single-food and multiple-food meals.

Experiment 1

Experiment 1 was an initial attempt to determine the effects of CCK on food intake in single-food and multiple-food meals. The two independent variables were the nature of the injection and the nature of the meal that was available. There were two levels of the injection condition: 0 and 8 \text{ug/kg} CCK injected intraperitoneally (i.p.). There were four meal types. In the three single-food meals, either milk chocolate (MC), peanut butter diet (PB), or shortcake cookie (SC) was presented. In the multiple-food meal, all three of these foods were presented simultaneously. The dependent variable was the amount of food consumed during a 30-min test meal.

The hypotheses for Experiment 1 were as follows:

**Hypothesis 1.1.** When the foods in a multiple-food meal are presented simultaneously, intake in the multiple-food meals will be greater than intake in single-food meals.

**Hypothesis 1.2.** Administration of exogenous CCK-8 will cause a decrease in food consumption.

**Hypothesis 1.3.** According to the recent-foods hypothesis, CCK-8 will be less effective at decreasing food intake in multiple-food meals than in single-food meals. In contrast, according to the all-foods hypothesis, CCK-8 will be equally effective in reducing food intake in multiple-food and single-food meals.
Methods

Subjects

Eleven male rats of the Wistar strain were used as subjects. At the start of the experiment the rats weighed approximately 500 - 650 g. The rats were not naive, as they had been previously utilized in pilot studies in which they received powdered Purina Rodent Chow (# 5001) to which various flavours had been added (garlic, saccharin, vanilla, orange, and lemon).

Throughout the experiment, rats were housed in individual hanging metal cages (24.4 cm x 40.8 cm x 16.8 cm), with mesh floor and front, in conditions of controlled temperature and lighting. There was a 13 hour light: 11 hour dark cycle, with lights on at 8:00 am and lights off at 9:00 pm. The temperature was maintained between 20-22 °C. Water was always available, and rats had continuous access to pelleted Purina Rodent Chow, except as described as below.

Materials

There were three foods utilized in this experiment, all having similar caloric and fat content. The foods were Alprose swiss milk chocolate (MC; 4.97 kcal/gram, 44% kcal from fat), Peek Freans' shortcake cookies (SC; 5.1 kcals/g, 43% kcal from fat), and a peanut butter diet (PB; 5.0 kcal/g, 41% kcals from fat) comprised of 100g of Smooth Kraft Light Peanut Butter and 64g of icing sugar. These foods were used because they are isocaloric and similar in fat content, so that the amount of energy derived from the foods would not be the basis for the animal selecting the particular food. The foods differed substantially in taste, texture, and odour, making it likely that greater food intake would be observed in multiple-food than in single-food meals (Rolls, B.J. et al., 1981).
During test meals, the MC and the SC were presented on the floor of the home cage, and PB was presented in a glass cup (5 cm × 6 cm) cemented to a ceramic tile (7 cm × 7 cm). Food intake was measured using an electronic balance (+ 0.01 g; model TR-2102; Denver Instrument Company).

There were two levels of the injection condition: 0 and 8 μg/kg doses of CCK. The CCK solution was made by mixing the synthetic C-terminal octapeptide of cholecystokinin (Sigma-Aldrich Canada Ltd., Oakville ON) with 0.15 M saline to create a solution with a concentration of 8 μg/ml. The 0 μg/kg CCK dose consisted of 0.15 M saline. The 8 μg/kg dose of CCK was used because in past research it has been shown to reliably reduce food consumption (Moran et al., 1998). Injections were administered i.p. using a 1-ml syringe with a 5/8", 25-gauge needle.

Procedure

Before the study commenced, the rats were pre-exposed to each of the three foods for one 24-hr session and one 30-min session. This was done to familiarize the rats with the three food items and with the 30-min test meal. A 30-min test meal was selected because CCK has been shown to have short lasting effects (Kulkosky et al., 1989) and because rats tend to consume most of the food in a meal within this time period. The rats were also familiarized with the injection procedure via four mock i.p. injections administered four days in a row, two months prior to the experiment. Rats received another two mock injections over a two-day period, a week-and-a-half before the experiment.

During the experiment each rat was tested under eight conditions, with each of the four meal types being paired with each of the two injection conditions. In the three single-food meals,
rats were given between 12.5 - 14.5g of one of the three foods for 30 min. In the multiple-food meal, all three foods (between 12.5 - 14.5g of each) were available throughout the 30-min test meal. The CCK solution was injected in a volume of 1ml/kg to yield a dose of 8 μg/kg CCK, and 0.15M saline was administered in a volume of 1ml/kg (0 μg/kg CCK). The rats were tested on eight consecutive days during the late afternoon, between 4:00 and 7:00 pm.

Before the start of testing on a given day, the food for all the rats was weighed and set out in preparation for the study. During the experiment, each rat was removed from its home cage, injected i.p. with either a 0 or 8 μg/kg dose of CCK, and then placed in a holding container. While the rat was in the holding container, the rat chow was removed from the home cage, and the rat was then placed back in its home cage. Next, the appropriate food(s) was (were) placed in the rat’s cage five minutes after the injection was administered. Two sheets of white paper were placed under the cage to collect any spillage. The food remained in the cage during the 30-minute testing period, and it was then removed from the home cage and re-weighed. Rat chow was returned to the home cage shortly after the end of testing. Water was freely available throughout the testing period, but intake was not measured. The rats were weighed on days 1 and 5 of the study.

Analyses

The data were analyzed using repeated-measures analyses of variance (ANOVA) and planned comparisons were analyzed by protected paired-sample t-tests. The t-tests were protected using the modified Bonferroni test which involves multiplying the chosen alpha by the degrees of freedom, dividing this value by the number of planned comparisons, and then using this computed
value as the alpha for each of the planned comparisons (Keppel, 1991). It was planned that every possible comparison would be made, with the stipulation that the two means being compared could differ along only one of the independent variables.

Results

Food Intake in the Average of the Single-food Conditions and in the Multiple-Food Condition

The first analysis focussed on the food intake in the multiple-food meal versus the average food intake of the single-food meals. The data were analyzed using a $2 \times 2$ repeated-measures ANOVA, with two levels of injection (0 and 8 $\mu$g/kg doses of CCK) and two meal types (single-food and multiple-food). This analysis revealed that there were significant main effects of meal type ($F_{(1,10)} = 30.55, p < 0.001$; Figure 1) and injection condition ($F_{(1,10)} = 291.16, p < 0.001$; Figure 2). Rats consumed 50% more food in the multiple-food condition than in the average of the single-food conditions. Overall, rats consumed 67% less food when injected with the 8 $\mu$g/kg dose of CCK than when injected with saline.

The meal type $\times$ injection interaction was not significant ($F_{(1,10)} = 4.91, p = 0.051$; Figure 3). As a follow up to this analysis, paired-sample $t$-tests ($\alpha = 0.0125$; modified Bonferroni test) were carried out to make all possible comparisons between meal types within each level of the injection condition and between levels of injection within each level of meal type. The injection of CCK reduced food intake by 70% and 66% in the single-food and multiple-food meals, respectively. Thus, the 8 $\mu$g/kg dose of CCK caused a similar percentage decrease in food intake in both meal types.
Food Intakes in MC, PB, SC, and Multiple-food Conditions

It should be noted that comparing the size of the multiple-food meal to the mean size of the single-food meals ignores the possibility that certain foods may be more acceptable to rats than others. For example, if the food in a single-food meal is particularly well accepted, consumption may approach that seen in the multiple-food meals. On the other hand, if the food in a single-food meal is not well accepted, intake will be low, and this low intake will pull down the mean intake when single-food meals are averaged together. Accordingly, comparing the size of each of the single-food meals to the size of the multiple-food meal will provide a more detailed examination of the differences in intake between single- and multiple-food meals.

To further investigate the effects of dietary variety on food consumption, the data were analyzed using a $2 \times 4$ repeated-measures ANOVA, with two levels of the injection condition (0 & 8 $\mu$g/kg doses of CCK) and four meal types (MC, PB, CS, and multiple-food). There were significant main effects of meal type ($F_{(3,30)} = 18.27, p < 0.001$; Figure 4) and injection condition ($F_{(1,10)} = 506.66, p < 0.001$; Figure 5). Dietary variety increased food intake by 18, 42, and 118% compared to the intake of SC, PB, and MC, respectively. Therefore, dietary variety is more effective in increasing food intake in comparison to certain single-food meals over others. The 8 $\mu$g/kg dose of CCK reduced food intake by 69%.

The meal type $\times$ injection interaction was significant ($F_{(3,30)} = 7.63, p = 0.003$; Figure 6). An examination of the simple effect of meal type for animals in the saline condition revealed a significant effect ($F_{(3,30)} = 16.26, p < 0.001$). Paired sample $t$-tests ($\alpha = 0.025$) were conducted on all possible comparisons of the four diets within the saline condition. These analyses revealed that
food intake was significantly greater in the multiple-food meal than in the MC meal ($t_{1,10} = -5.50, p < 0.001$) and PB meal ($t_{1,10} = -3.03, p = 0.008$), but not greater than in the SC meal ($t_{1,10} = -1.81, p = 0.100$).

The simple effect of meal type for animals in the CCK condition was also significant ($F_{3,30} = 7.47, p = 0.001$). Paired-sample $t$-tests ($\alpha = 0.025$) were conducted on all possible comparisons of the four meal types within the CCK level of the injection condition. Results indicated that food consumption was significantly different only between the MC and multiple-food conditions ($t_{1,10} = -4.39, p = 0.001$), with more food being consumed in the multiple-food meal. Therefore, in the CCK condition food intake in the multiple-food meal was not significantly greater than the amount consumed in the PB and SC meals.

Paired-sample $t$-tests ($\alpha = 0.025$) were conducted on all possible comparisons between the two levels of injection within each meal type. The $t$-tests revealed that food consumption of each of the diets was significantly different from one level of injection to the other. The 8 $\mu$g/kg dose of CCK decreased the amount of MC consumed by 70% ($t_{1,10} = -5.78, p < 0.001$), PB by 68% ($t_{1,10} = -14.27, p < 0.001$), SC by 72% ($t_{1,10} = -13.39, p < 0.001$), and the multiple-food meal by 66% ($t_{1,10} = -9.65, p < 0.001$). This pattern of results indicates that the 8 $\mu$g/kg dose of CCK caused a similar percentage decrease in food intake across all meal types.

Comparison of Food Intakes within the Multiple-Food Meal

Recall that the multiple-food meal was significantly larger than the mean of the single-food meals. This difference may be due not to the enhancement of intake by dietary variety, but rather to a strong preference for one of the foods that is available during the multiple-food meal. The
multiple-food meal is comprised of MC, PB, and SC, and it is possible that rats could consume only one of the three foods during the multiple-food meal. If this was the case, intake could still be greater in the multiple-food meal than in the average of the single-food meals, but the increased intake would be due to the preference for one of the three foods and not due to a variety effect. An examination of the intakes of the three distinct foods within the multiple-food meal makes it possible to determine whether it is dietary variety or a preference for one of the three foods that is enhancing food intake. In addition, it can be used to determine whether CCK has differential effects on the different foods within the meal.

A 2 × 3 repeated-measures ANOVA was conducted on the intake of the individual foods within the multiple-food meal, with two levels of injection (0 & 8 μg/kg doses of CCK) and three levels of food (MC, PB, & SC). There were significant main effects of food type ($F_{(2,20)} = 13.56, p < 0.001$; Figure 7) and injection condition ($F_{(1,10)} = 93.15, p < 0.001$; Figure 8). Intake of SC was greater than that of MC ($t_{(1,10)} = -4.93, p = 0.001$) and PB ($t_{(1,10)} = -3.18, p = 0.010$), but intakes of MC and PB did not differ significantly ($t_{(1,10)} = -1.88, p = 0.089$). Twelve percent, 27%, and 61% of total intake of the multiple-food meal was derived from MC, PB, and SC, respectively. The 8 μg/kg dose of CCK inhibited food intake by 66%.

The food type × injection interaction was significant ($F_{(2,20)} = 4.14, p = 0.036$; Figure 9). There was a significant simple effect of meal type in both the saline ($F_{(2,20)} = 9.68, p = 0.002$) and the CCK conditions ($F_{(2,20)} = 8.73, p = 0.006$). Within the saline condition, 13%, 26%, and 62% of total intake was derived from MC, PB, and SC, respectively. Within the CCK condition, 6.5%, 32.5%, and 61% of total food intake was derived from MC, PB, and SC, respectively. Therefore,
in both the saline and CCK conditions, MC was the least preferred food and SC was most preferred. This result suggests that the enhancement of intake of the multiple-food meal compared to the mean of the single-food meals and the three single-food meals individually was due to the effects of dietary variety and not simply to a preference for one of the foods. Thus, it is clear that rats consumed some of each of the three foods within the multiple-food meal, and that even the most highly preferred food (SC) accounted for less than two-thirds of total intake.

Paired-sample t-tests ($\alpha = 0.017$) were also conducted within each food type. CCK inhibited the intake of SC by 66% ($t_{(1,10)} = 4.87, p = 0.001$), PB by 56% ($t_{(1,10)} = 2.14, p = 0.058$, n.s.), and MC by 83% ($t_{(1,10)} = 2.05, p = 0.068$). Therefore, CCK significantly decreased the intake of only SC within the multiple-food meal. Despite this finding, there seems to have been a floor effect, as CCK decreased the intake of both PB and MC by quite high percentages. Although the percentage decrease in intake was quite high, the decrease in gram intake was relatively small because the rats did not consume large quantities of MC and PB within the multiple-food meal. Therefore, this floor effect may explain why the reductions in intake of MC and PB were not significant.

**Analysis of Kilocalorie Data**

Because the energy densities of the three foods used were similar, but not identical, the data were transformed from gram intake to kilocalorie intake. Analyses were conducted on the kilocalorie data, and the results were very similar to the those presented above. Because the same conclusions were reached, results of the analyses of the kilocalorie data will not be presented here.
Discussion

In Experiment 1, the mean intake of the multiple-food meal was 50% greater than the combined mean of the three single-food meals which supports hypothesis 1.1. This compares to a previous report of 26% greater food intake in a multiple-food than a single-food meal (Rolls, B.J. et al., 1982). Thus, the experimental procedure of providing dietary variety was effective in increasing the size of the multiple-food meal compared to the mean of the single-food meals.

The results also revealed that SC was the most preferred and MC was the least preferred of the three foods. This was the case with respect to intake within both single-food and multiple-food meals. The multiple-food meal led to enhanced food intake compared to all three single-food meals.

The injection condition also affected food consumption, with an 8 µg/kg dose of CCK inhibiting food intake by approximately 67% which supports hypothesis 1.2. This compares to a previous finding that a 8 µg/kg dose of CCK inhibited food intake by 67% (Moran et al., 1998). Furthermore, this dose of CCK inhibited food intake by similar percentages in both single-food and a multiple-food meals. Therefore, meal type (single-food and multiple-food) did not moderate the effects of CCK. It was hypothesized that CCK-8 would be less effective in decreasing food intake when a multiple-food meal as opposed to a single-food meal is available for consumption. This hypothesis was based on the recent-foods hypothesis that postulates that CCK reduces the palatability of only recently consumed foods. The finding that CCK did not differentially affect the intake of the two meal types does not provide support for the recent-foods hypothesis. However, it is consistent with the all-foods hypothesis which predicts that CCK will affect the intake of
single-food and multiple-food meals to a similar extent, with no interaction occurring between CCK and dietary variety. Thus, this finding suggests that CCK enhances satiety in a very general way and reduces the palatability of all foods, not only of those foods that have recently been consumed.

Experiment 2

A limitation of Experiment 1 is that only one dose of CCK was used. It is well known that there are dose-dependent effects of CCK (Moran et al., 1998). Moran et al. (1998) demonstrated that doses of 1-16 µg/kg of CCK produce decreases in intake of a single food ranging from 29% to 71% with the magnitude of decreases directly related to the dose of CCK. The 8 µg/kg dose of CCK reduce intake by 67% (Moran et al., 1998). Because a 8µ/kg dose of CCK was used in Experiment 1, the inhibitory effects of this dose may have been great enough to cause uniform suppression of food intake, regardless of meal type. The use of lower doses of CCK may permit the discovery of any differences in the effectiveness of CCK in single-food and multiple-food meals. To investigate this possibility, Experiment 2 was conducted.

In Experiment 2, the same general procedures were used as in Experiment 1. However, lower doses of CCK were used. The hypotheses for Experiment 2 were as follows:

**Hypothesis 2.1.** When the foods in a multiple-food meal are presented simultaneously, intake in the multiple-food meals will be greater than intake in single-food meals.

**Hypothesis 2.2.** Administration of exogenous CCK-8 in a range of doses will cause dose-dependent decreases in food consumption.
Hypothesis 2.3. According to the recent-foods hypothesis, CCK-8 will be less effective at decreasing food intake in multiple-food meals than in single-food meals. In contrast, according to the all-foods hypothesis, CCK-8 will be equally effective in reducing food intake in multiple-food and single-food meals.

Methods

Subjects, Housing, and Materials

In Experiment 2, 14 naive male Wistar rats were utilized. At the start of the experiment they weighed approximately 500 - 650 g. The housing conditions and the foods used were the same as those described in Experiment 1. The CCK doses were 0, 2, and 4 μg/kg of CCK.

Procedure

Before the study commenced, the rats were pre-exposed to each of the three foods for one 24-hr session and one 30-min session. Rats received a mock injection on each of the two days prior to the commencement of Experiment 2. During the experiment, rats were tested under twelve conditions, with each of four meal types (MC, PB, SC, and multiple-food) being paired with each of three levels of injection condition (0, 2, & 4 μg/kg doses of CCK). The general procedures were similar to those of Experiment 1. The CCK solution had a concentration of 4 μg/ml, so it was administered in volumes of 0.5 and 1 ml/kg i.p. to produce 2 and 4 μg/kg doses of CCK, respectively. The 0 μg/kg dose of CCK was 0.15M saline administered in a volume of 1ml/kg i.p. The rats were weighed on days 1, 5, and 9. Testing occurred on twelve consecutive days, between 4:00 and 7:30 pm.
Results

Food Intake in the Average of the Single-food Conditions and in the Multiple-Food Condition

The first analysis focussed on the food intake in the multiple-food meal versus the average food intake of the single-food meals. The data were analyzed using a $3 \times 2$ repeated-measures ANOVA, with three levels of injection condition (0, 2, & 4 μg/kg doses of CCK) and two meal types (single-food and multiple-food). This analysis revealed that there were significant main effects of meal type ($F_{(1,13)} = 30.77, p < 0.001$; Figure 10) and injection condition ($F_{(2,26)} = 37.92, p < 0.001$; Figure 11). Rats consumed 45% more in the multiple-food meal compared to average intake of the single-food meals. The 2 and 4 μ/kg doses of CCK inhibited food intake by 42% ($t_{(1,13)} = 7.96, p< 0.001$) and 60% ($t_{(1,13)} = 8.24, p < 0.001$), respectively.

The diet $\times$ injection interaction approached significance ($F_{(2,26)} = 3.20, p = 0.067$; Figure 12). As a follow up to this analysis, paired-sample $t$-tests ($\alpha = 0.008$) were conducted to make all possible comparisons between the intakes of the meal types within each level of the injection condition and between the injection conditions within each meal type. The 2 μg/kg dose of CCK inhibited food intake by 43% and 41% in the single-food and multiple-food meals, respectively. The 4 μg/kg dose of CCK suppressed food intake by 58% and 61% in the single-food and multiple-food meals, respectively. Thus, the 2 μg/kg dose of CCK caused a similar percentage decrease in food intake in both types of meal. The same was true for the 4 μg/kg dose of CCK.

Food Intakes in MC, PB, SC, and Multiple-food Conditions

To further investigate the effects of dietary variety on food intake, the data were analyzed using a $3 \times 4$ repeated-measures ANOVA, with three levels of injection condition (0, 2, & 4
\(\mu g/kg\) doses of CCK) and four meal types (MC, PB, SC, and multiple-food). There were significant main effects of meal type \((F(3,39) = 17.04, p < 0.001;\) Figure 13) and injection condition \((F(2,26) = 46.58, p < 0.001;\) Figure 14). The multiple-food meal increased food intake between 10\% and 133\%, although the 10\% increased intake of the multiple-food meal over SC was not significant \((t(1,13) = -1.34, p = 0.204)\). Overall, in the single-food condition SC intake was greater than PB, and PB was greater than MC. Intake in the multiple-food condition was greater than intake of PB and of SC, but not greater than the intake of SC. The CCK injections also affected the amount of food consumed by the rats. Overall, 2 and 4 \(\mu g/kg\) doses of CCK reduced food intake by approximately 43\% \((t(1,13) = 8.60, p < 0.001)\) and 59\% \((t(1,13) = 8.97, p < 0.001)\) respectively. The meal type \(\times\) injection interaction was not significant \((F(6,78) = 1.77, p = 0.155;\) Figure 15).

Comparison of Food Intakes within the Multiple-Food Meal

As stated previously, in the single-food conditions, rats consumed more SC than PB, and more PB than MC. As in Experiment 1, rats consumed more food in the multiple-food meal than in the PB and MC meals, but in Experiment 2 the SC and the multiple-food meals were similar in size. Therefore, the differences in intake between the multiple-food meal and the PB and MC meals may be due not to the enhancement of intake by dietary variety, but rather to a preference for SC. Thus, it is important to look at the intakes of the three foods within the multiple-food meal to determine if it is dietary variety or a preference for SC that is enhancing food intake in the multiple-food meal.
A 3 × 3 repeated measures ANOVA was conducted on the intake of the individual foods within the multiple-food meal condition, with three levels of food (MC, PB, & SC) and three levels of injection (0, 2, & 4 μg/kg doses of CCK). There were significant main effects of the food condition ($F_{(2,26)} = 8.41, \ p = 0.006$; Figure 16) and injection condition ($F_{(2,26)} = 22.46, \ p < 0.001$; Figure 17). Overall, intakes of both SC ($t_{(1,13)} = -5.91, \ p < 0.001$) and PB ($t_{(1,13)} = -2.73, \ p = 0.017$) were greater than intake of MC, but intake of SC and PB did not differ ($t_{(1,13)} = -1.18, \ p = 0.260$). Overall, 8%, 37%, and 55% of total intake of the multiple-food meal was derived from MC, PB, and SC, respectively. The 2 and 4 μg/kg doses of CCK decreased food intake by 41% and 61%, respectively.

The food type × injection interaction was significant ($F_{(4,52)} = 4.70, \ p = 0.012$; Figure 18). Paired-sample $t$-tests ($\alpha = 0.011$) were conducted within each level of the injection condition. Within the saline condition, 10%, 29%, and 61% of total intake was derived from MC, PB, and SC respectively. Within the 2 μg/kg dose of CCK condition, 8%, 48%, and 44% of total intake was derived from MC, PB, and SC, respectively. Within the 4 μg/kg dose of CCK condition, 2%, 44%, and 54% of total intake was derived from MC, PB, and SC, respectively. Therefore, in all the injection conditions, MC was less preferred than SC. These results suggests that the enhancement of intake of the multiple-food meal compared to the MC and PB meals was due to the effects of dietary variety and not simply to a preference for one of the foods. As in Experiment 1, it is clear that rats consumed some of each of the three foods within the multiple-food meal, and that even the most highly preferred food (SC) accounted for less than two-thirds of total intake.
Paired-sample $t$-tests ($\alpha = 0.011$) were also conducted within each food type. The only food whose intake was significantly affected by CCK was SC. SC consumption was significantly greater in the saline condition than in the 2 $\mu g/kg$ dose of CCK ($t_{(1,13)} = 5.33, p < 0.001$) and 4 $\mu g/kg$ dose of CCK ($t_{(1,13)} = 5.89, p < 0.001$) conditions. Therefore, CCK reduced the intake of SC, but not of either PB or MC within the multiple-food meal.

Discussion

In Experiment 2 the mean intake of the multiple-food meal was 45% greater than the combined mean of the three single-food meals, which is similar to the value (50%) reported in Experiment 1 and supports hypothesis 2.1. This compares to a previous report of 26% greater food intake in a multiple-food than a single-food meal (Rolls, B.J. et al., 1982). As in Experiment 1, the experimental procedure of providing dietary variety was effective in increasing the size of the multiple-food meal compared to the mean of the single-food meals.

The results also revealed that SC was more preferred than MC. This was the case with respect to intake within both single-food and multiple-food meals. The multiple-food meal led to enhanced food intake compared to the MC and PB meals, but not compared to the SC meal.

The injection condition also affected food consumption, with 2 and 4 $\mu g/kg$ doses of CCK inhibiting food intake by approximately 42% and 60%, respectively. This finding supports hypothesis 2.2. This compares to a previous finding that 2 and 4 $\mu g/kg$ doses of CCK inhibited food intake by 43% and 62% (Moran et al., 1998). In Experiment 1, it was reported that an 8 $\mu g/kg$ dose of CCK inhibited food intake by 67% which is comparable to Moran et al.'s (1998) finding that an 8 $\mu g/kg$ dose of CCK inhibited intake by 67%. Furthermore, these doses of CCK
inhibited food intake by similar percentages in both single-food and a multiple-food meals. As in Experiment 1, meal type (single-food and multiple-food) did not moderate the effects of CCK. The recent-foods hypothesis predicted that CCK-8 would be less effective in decreasing food intake in the multiple-food meal than in the single-food meals. As was the case in Experiment 1, Experiment 2 did not provide support for the recent-foods hypothesis, but results were consistent with the all-foods hypothesis, suggesting that CCK enhances satiety in a very general way and reduces the palatability of all foods, not only those that have been recently consumed.

One noteworthy limitation of both Experiment 1 and 2 was that in some cases rats consumed all of the food in one or more of the single-food meals, and in some cases they consumed all of one of the foods available in the multiple-food meals. In both types of situation, SC was the food that was most often completely consumed. This complete consumption of a food usually occurred when the injection prior to food presentation was saline, but it also occurred on occasion with injection of other doses of CCK. Table 1 provides a detailed summary of the instances in which animals ate all of a particular food in Experiments 1 and 2. Because animals sometimes consumed all of a particular food, the measured food intake may underestimate what animals would have eaten if sufficient amount of food had been available to them. This underestimation means that the results of Experiments 1 and 2 are difficult to interpret. Accordingly, Experiment 3 was conducted with more food available during each meal to get a more accurate reflection of the effects of CCK and dietary variety on food intake.
Experiment 3

Because it was not possible in Experiments 1 and 2 to determine how much rats might have consumed if they had not run out of food on certain occasions, Experiment 3 was conducted. In Experiment 3, all four doses of CCK (0, 2, 4, and 8 μg/kg) from Experiments 1 and 2 were used, and the amount of each type of food placed in the cages during the 30-min test meals was increased from 14 to 28 g.

The hypotheses for Experiment 3 were as follows:

**Hypothesis 3.1.** When the foods in a multiple-food meal are presented simultaneously, intake in the multiple-food meals will be greater than intake in single-food meals.

**Hypothesis 3.2.** Administration of exogenous CCK-8 in a range of doses will cause dose-dependent decreases in food consumption.

**Hypothesis 3.3.** According to the recent-foods hypothesis, CCK-8 will be less effective at decreasing food intake in multiple-food meals than in single-food meals. In contrast, according to the all-foods hypothesis, CCK-8 will be equally effective in reducing food intake in multiple-food and single-food meals.

**Methods**

**Subjects, Housing and Materials**

In Experiment 3, 16 naive male Wistar rats were utilized. At the start of the experiment
they weighed approximately 330 - 375 g. The housing conditions were the same as in Experiments 1 and 2, and once again the foods were MC, PB, and SC.

Procedure

Before the study commenced, the rats were pre-exposed to each of the three foods for one 24-hr session and one 30-min session. Also, rats received a mock injection on each of two consecutive days, starting three days prior to the commencement of Experiment 3. The rats were tested under sixteen conditions, with each of four meal types (MC, PB, SC, and multiple-food) being paired with each of four levels of injection (0, 2, 4, & 8 μg/kg doses of CCK administered i.p.). The general procedures were similar to those of the Experiment 1 and 2. However, the rats received an amount of food in each meal that insured that they would never consume all of the presented food. Thus, each rat received between 25.5 - 28.5 g of food in the single-food meals and between 25.5 - 28.5 g of each food in the multiple-food meal. Rats were weighed on each day of testing. Testing occurred every second day over a four-week period, between 2:30 and 5:00 pm.

Results

Food Intake in the Average of the Single-food Conditions and in the Multiple-Food Condition

The first analysis focussed on the food intake in the multiple-food meal versus the average food intake of the single-food meals. The data were analyzed using a 4 × 2 repeated-measures ANOVA, with four levels of injection condition (0, 2, 4, & 8 μg/kg doses of CCK) and two meal types (single-food and multiple-food). This analysis revealed that there were significant main effects of meal type (F(1,15) = 35.94, p < 0.001; Figure 19) and injection condition (F(3,45) = 26.92, p
< 0.001; Figure 20). Rats consumed 43% more food in the multiple-food meals that in the single-food meals. Overall, the 2, 4, and 8 μg/kg doses of CCK significantly reduced food intake by 23% ($t(4.15) = 4.21, p = 0.001$), 36% ($t(4.15) = 5.21, p < 0.001$), and 50% ($t(4.15) = 8.10, p < 0.001$), respectively.

The meal type $\times$ injection interaction was not significant ($F(3.45) = 2.55, p = 0.087$; Figure 21). Paired-sample $t$-tests ($\alpha = 0.0125$) were conducted to make all possible comparisons between the intakes of the meal types within each injection condition and all possible comparisons between food intake in the injection conditions within each meal type. Rats consumed more of the multiple-food meal than the single-food meal in all of the injection conditions, except for the 8 μg/kg dose of CCK ($t(1.15) = 1.41, p = 0.179$).

$T$-tests ($\alpha = 0.0125$) also demonstrated that in the multiple-food meal condition, 2, 4, and 8 μg/kg doses of CCK significantly decreased food intake by 27% ($t(1.15) = 3.12, p = 0.007$), 39% ($t(1.15) = 4.13, p = 0.001$), and 53% ($t(1.15) = 6.25, p < 0.001$), respectively. In the single-food meal condition, the 2 μg/kg dose of CCK did not significantly decrease food intake, but the 4 and 8 μg/kg doses of CCK inhibited food intake by 31% ($t(1.15) = 4.08, p = 0.001$) and 45% ($t(1.15) = 6.83, p < 0.001$), respectively. Therefore, for the most part, the three doses of CCK reduced food intake by a similar amount in the multiple- and single-food meals.

**Food Intakes in MC, PB, SC, and Multiple-food Meals**

To further investigate the effects of dietary variety on food intake, the data were analyzed using a $4 \times 4$ repeated-measures ANOVA, with four levels of injection (0, 2, 4, & 8 μg/kg doses of CCK) and four meal types (MC, PB, SC, and multiple-food meals). There was a significant main
effect of meal type ($F_{(3,45)} = 25.12, \ p < 0.001$; Figure 22), with dietary variety significantly increasing intake by 22 to 107% compared to the single-food meals. PB and SC meals did not differ significantly in size, and both were significantly greater in size than MC meals (PB: $t_{(1,15)} = -7.84, \ p < 0.001$; SC: $t_{(1,15)} = -5.01, \ p < 0.001$). The size of the multiple-food meal exceeded that of the MC, PB, and SC meals by 107% ($t_{(1,15)} = -7.58, \ p < 0.001$), 25% ($t_{(1,15)} = -3.40, \ p = 0.004$), and 22% ($t_{(1,15)} = -3.24, \ p = 0.005$), respectively.

There was also a significant main effect of injection condition ($F_{(3,45)} = 29.87, \ p < 0.001$; Figure 23). Overall, there was a clear dose-dependent effect of CCK on food intake, with 2, 4, and 8 \( \mu g/kg \) doses of CCK significantly reducing food intake by approximately 20% ($t_{(1,15)} = 4.01, \ p = 0.001$), 33% ($t_{(1,15)} = 5.28, \ p < 0.001$), and 48% ($t_{(1,15)} = 8.51, \ p < 0.001$), respectively.

The diet \( \times \) injection interaction was significant ($F_{(9,135)} = 2.40, \ p = 0.039$; Figure 24). In further analyses, it was determined that the enhancing effect of dietary variety on meal size is greatest in the saline condition, and it decreases as the dose of CCK becomes larger. Paired-sample $t$-tests ($\alpha = 0.006$) revealed that in saline-injected rats, the multiple-food meal enhanced consumption by 27% or more. Within the 4 \( \mu g/kg \) dose of CCK condition, the only significant difference in food intake was less intake of MC than the multiple-food meal ($t_{(1,15)} = -4.19, \ p = 0.001$). Also, with the 8 \( \mu g/kg \) dose of CCK condition, no significant differences in intake were found between any of the four meal types. This suggests that this high dose of CCK may have such a large suppressive effect that other factors that have been shown to affect food, such as dietary variety, may be overshadowed.
Next, paired-sample \( t \)-tests (\( \alpha = 0.006 \)) were conducted on all possible comparisons among the four injection conditions within each meal type. It was determined that CCK was at least as effective in decreasing food intake in multiple-food meals as in single-food meals, and maybe even more so. Thus, all these doses of CCK significantly reduced meal size only in the multiple-food meal, and the CCK-induced decreases on food intake were largest in the multiple-food meal. These analyses also revealed that within all the meal types, except the MC meal, there were dose-dependent effects of CCK. MC was the least consumed food, and within the MC meal no significant differences in intake were found among any of the injection conditions. This suggests CCK may be less effective at decreasing intake of foods that are consumed in smaller amounts than of foods that are consumed in greater quantities.

Comparison of Food Intakes within the Multiple-food Meal

A \( 3 \times 4 \) repeated-measures ANOVA was conducted on the intake of the individuals foods within the multiple-food meal, with three levels of food (MC, PB, & SC) and four levels of injection (0, 2, 4, & 8 \( \mu g/kg \) doses of CCK). There were significant main effects of food type (\( F_{(2,30)} = 12.87, p < 0.001; \) Figure 25) and injection condition (\( F_{(3,45)} = 14.58, p < 0.001; \) Figure 26). There was significantly greater intake of PB than MC (\( t_{(1,15)} = -3.06, p = 0.008 \)), of SC than MC (\( t_{(1,15)} = -4.37, p = 0.001 \)), and of SC than PB (\( t_{(1,15)} = -2.58, p = 0.021 \)). Nineteen percent, 33%, and 48% of total intake was derived from MC, PB, and SC, respectively. Therefore, MC was the least preferred food and SC the most preferred food. These results suggests that the enhancement of intake of the multiple-food meal compared to the single-food meals was due to the effects of dietary variety and not simply to a preference for one of the foods. As in
Experiments 1 and 2, it is clear that rats consumed some of each of the three foods within the multiple-food meal, and that even the most highly preferred food (SC) accounted for less than one-half of total intake. The 2, 4, and 8 $\mu$g/kg doses of CCK reduced intake by 26, 38, and 53%, respectively. The food type $\times$ injection interaction was not significant ($F_{(3,45)} = 0.70, p = 0.594; \text{Figure 27}$).

**Discussion**

In Experiment 3 the mean intake of the multiple-food meal was 43% greater than the combined mean of the three single-food meals (supporting hypothesis 3.1), which is similar to the values reported in Experiments 1 and 2 (50% and 45%, respectively). These values compare to previous reports of dietary variety increasing food intake by 23% (Clifton et al., 1987) and 26% (Rolls, B.J. et al., 1982). As in Experiments 1 and 2, giving access to dietary variety was effective in increasing the size of the multiple-meal compared to the mean of the single-food meals.

The injection condition also influenced food intake in a dose-dependent fashion, with 2, 4, and 8 $\mu$g/kg doses of CCK reducing intake by 23%, 36%, and 50%, respectively (supporting hypothesis 3.2), which is consistent with the findings from Experiment 2, that 2 and 4 $\mu$g/kg dose of CCK inhibited intake by 42% and 60%, respectively, and the finding from Experiment 1, that a 8 $\mu$g/kg dose of CCK inhibited intake by 67%. In previous research it was reported that 2, 4, and 8 $\mu$g/kg doses of CCK inhibited food intake by 43%, 62% and 67%, respectively (Moran et al., 1998). Furthermore, the results suggest that the inhibitory effects of CCK on food intake may be similar in single-food and multiple-food meals, as these doses of CCK inhibited food intake by similar percentages in both types of meals. As in Experiments 1 and 2, meal type does not appear
to be an important factor in determining the effectiveness of CCK. Therefore, the results do not support the prediction based on the recent-foods hypothesis that CCK-8 would be less effective in decreasing food intake in a multiple-food meal than in a single-food meal. The results are consistent with the prediction of the all-foods hypothesis, suggesting that CCK may reduce the palatability of foods in general, and not just the palatability of recently consumed foods. Also, the higher the dose of CCK administered, the less effective dietary variety was at enhancing intake. This suggests that higher doses of CCK may have such large suppressive effects that other factors that have been shown to affect food intake, such as dietary variety, are overshadowed.

The rat's acceptance of the meal appears to be a more important factor than whether the meal is composed of a single food or multiple foods in determining the inhibitory effectiveness of CCK. Thus, when intake is lower, as it is in MC meal, CCK appears to be less effective. In Experiment 3, for example, the MC meal was consumed in small quantities and none of the doses of CCK were effective in reducing intake of this meal. In contrast, the PB and SC meals were more readily consumed and CCK significantly reduced the intake of these single-food meals. Therefore, the quantity of food consumed may be an important factor in determining the effectiveness of CCK in reducing food intake.

As was found in Experiment 1, MC was the least preferred food, and SC the most preferred food. These preferences could have an impact on total food intake if the foods within the multiple-food meal were presented successively rather than simultaneously. In its natural environment an animal may come across a variety of foods in quick succession, rather than simultaneously. Accordingly, it is important to study the effects of dietary variety when foods are
presented successively rather than simultaneously. When foods are encountered successively, the order in which the animal experiences these foods may also affect food intake. For example, encountering foods in an increasing order of preference versus a decreasing order of preference may result in a different pattern of consumption and may also affect the total amount of food consumed. Therefore, studying the effects of encountering foods in an increasing versus a decreasing order of preference may provide some insight into how preference and the order in which these foods are experienced play a role in feeding behaviour in an animal’s natural environment. Also, the effects of CCK on food intake may interact in a different manner with the effects of dietary variety, if the dietary variety is presented successively rather than simultaneously. Thus, Experiment 4 was conducted to examine 1) the effects of successive presentation of foods on food intake, 2) the effects of experiencing foods in increasing versus decreasing order of preference, and 3) the effects of CCK on food intake when foods are experienced in these two orders of preference.

Experiment 4

Experiment 3 helped determine that the inhibitory effects of CCK on food intake were similar for single-food and multiple-food meals when the foods in the multiple-food meal are presented simultaneously. Despite having found that the inhibitory effects of CCK were similar for both meal types, it is possible that the presentation style of the food in a multiple-food meal may have an impact on the effects of CCK. For example, CCK’s effects on food intake may differ when the foods in a multiple-food meal are presented successively rather than simultaneously. This is an important consideration because in an animal’s natural environment it may come across
foods in quick succession rather than simultaneously. Therefore it is important to examine the effects of CCK on food intake not only when foods are encountered simultaneously, but also when foods are encountered successively to provide a more complete understanding of what may be occurring in the natural world.

In a multiple-food meal in which the foods are presented simultaneously, the rat will most likely consume the most preferred food in the greatest quantity and the second most preferred food in the second largest quantity, and so on. Also, within a simultaneous meal the animal can switch back and forth between the foods throughout the meal, and this may occur because it has been previously demonstrated that stimulus-specific satiety can be relatively short lasting (Clifton et al., 1987). In contrast, when foods are presented successively, an animal may develop satiety toward the available food and stop eating it, and there will be no other food available for the animal to consume at that time. Therefore, because the animal cannot switch back and forth between foods when foods are presented successively, simultaneous presentation of foods in a multiple-food meal may lead to greater intake than successive presentation.

In a multiple-food meal where different foods are presented successively it would be expected that an animal would consume at least some food in each course, because although satiety may develop to the first food, satiety may be specific to that food and may not generalize to the other foods being presented due to differing stimulus properties of the foods. However, the order of presentation of the foods may affect the pattern and amount of food intake. If foods are presented in increasing order of preference versus decreasing order of preference the total amount of food consumed during the meal and/or the pattern of consumption may be affected. For
example, if foods are presented in a decreasing order of preference one might expect that food intake will be greatest in the first course and decrease with each consecutive course because the foods are becoming increasingly less preferred. Also, with each course there will be less of a tendency to eat as a result of the normal processes that lead to meal termination. On the other hand, if foods are presented in an increasing order of preference, the pattern of intake across courses may be quite different. Under these circumstances, intakes for the various courses may be more similar and, depending on the extent of the differences in preference, there may even been increases in intake from one course to the next. Thus, the order of presentation of foods could have a substantial effect on patterns of consumption and total intake in a multiple-food meal in which foods are presented successively.

The goal of Experiment 4 was to examine the effects of CCK on the intake of single-food and multiple-food meals, when the foods in both meal types are presented as three successive 10-min courses. Two multiple-food and one single-food meals were utilized. Because the previous experiments had indicated that SC was the most preferred food and MC was the least preferred food, the foods in the multiple-food meal were presented in either increasing order of preference (MC-PB-SC) or in decreasing order of preference (SC-PB-MC). The three courses of the single-food meal were SC-SC-SC. SC was chosen as the food for the single-food meal to determine whether dietary variety will increase food intake above the intake of the most preferred food. In order to examine the effects of a range of doses of CCK on food intake in each meal type, rats were injected with one of four doses of CCK (0, 2, 4, and 8 μg/kg) prior to the presentation of each of the three different meals.
The hypotheses for Experiment 4 were as follows:

**Hypothesis 4.1.** When the foods in a multiple-food meal are presented successively, intake in the multiple-food meals will be greater than intake in single-food meals.

**Hypothesis 4.2.** Administration of exogenous CCK-8 in a range of doses will cause dose-dependent decreases in food consumption.

**Hypothesis 4.3.** When foods are presented in an increasing order of preference intake will be greater than when foods are presented in a decreasing order of preference.

**Methods**

**Subjects, Housing and Materials**

In Experiment 4, 16 naive male Wistar rats were utilized. At the start of the experiment they weighed 250 - 310 g. The housing conditions were the same as in Experiments 1, 2, and 3 and once again the foods were MC, PB, and SC.

**Procedure**

Before the study commenced, the rats were pre-exposed to each of the three foods for one 24-hr session and one 30-min session consisting of three 10-min courses. Rats received a mock injection on each of two days within a five-day period before the commencement of Experiment 4. The procedure was similar to previous experiments except that the meals consisted of three 10-min courses, rather than one 30-min course. In both the single-food and multiple-food meals, between 25.5 - 28.5g of fresh food was placed in the home cage at the beginning of each course
and uneaten food was removed 10 min later. Three types of meal were used, with foods in the three successive 10-min courses as follows: (1) SC-SC-SC (single-food meal); (2) MC-PB-SC (multiple-food meal: increasing order of preference); (3) SC-PB-MC (multiple-food meal: decreasing order of preference). The rats were tested under twelve conditions, with each of the three meal types (SC-SC-SC, MC-PB-SC, and SC-PB-MC) being paired with each of four levels of injection (0, 2, 4, & 8 μg/kg doses of CCK administered i.p.). Rats were weighed on each day of testing. Testing occurred every second day over a twenty-four-day period, between 3:00 and 5:30 pm.

Results

Food Intakes in MC-PB-SC, SC-PB-MC, and SC-SC-SC Meals

In the first analysis, the data were analyzed using a 3 × 3 × 4 repeated-measures ANOVA, with 3 courses (courses 1, 2, and 3), 3 meal types (MC-PB-SC, SC-PB-MC, and SC-SC-SC conditions), and 4 levels of injection (0, 2, 4, & 8 μg/kg doses of CCK). There was a significant main effect of meal type ($F_{(3,30)} = 4.66, p = 0.024$; Figure 28). Compared to the SC-SC-SC meal, total food intake was significantly increased by 27% in the MC-PB-SC meal ($t_{(1,15)} = -2.55, p = 0.022$), but the 12% increase in the SC-PB-MC meal was not significant ($t_{(1,15)} = -1.44, p = 0.172$). There was also a significant main effect of course ($F_{(2,30)} = 6.94, p = 0.011$; Figure 29). Compared to course one, the 12% decrease in food intake in course two was not significant ($t_{(1,15)} = 0.90, p = 0.384$), but the 42% decrease in intake during course 3 was significant ($t_{(1,15)} = 6.80, p < 0.001$). Intake was not significantly different between course 2 and 3 ($t_{(1,15)} = 2.21, p < 0.043$). Finally, there was a significant main effect of injection condition ($F_{(3,45)} = 25.71, p < 0.001$; Figure
Overall, there was a clear dose-dependent effect of CCK on food intake, with doses of 2, 4, and 8 μg/kg CCK significantly reducing food intake by approximately 20% ($t_{(1,15)} = 4.01, p = 0.001$), 33% ($t_{(1,15)} = 5.28, p < 0.001$), and 48% ($t_{(1,15)} = 8.51, p < 0.001$), respectively.

The meal type × injection interaction was not significant ($F_{(6,90)} = 0.77, p = 0.54$; Figure 31). Therefore, the effects of CCK were similar in single-food and multiple-food meals.

The meal type × course interaction was significant ($F_{(4,60)} = 15.53, p < 0.001$; Figure 32). In the SC-SC-SC and SC-PB-MC meals, the general trend was for food intake to decrease across courses. Therefore, the patterns of intake in the SC-SC-SC meal resembles the pattern seen in a multiple-food meal in which foods are presented in decreasing order of preference. Quite a different trend was evident in the MC-PB-SC meal, with intake being greater in the second course than in the first course. This pattern of results suggests that food preferences may have a substantial impact both on total food intake and on the pattern of food intake within a multiple-food meal.

The course × injection interaction was also significant ($F_{(6,90)} = 6.12, p < 0.001$; Figure 33). Paired-sample $t$-tests ($\alpha = 0.008$) revealed that the three doses of CCK were more effective in inhibiting food intake during course one than during either of the other two courses. The 2 μg/kg ($t_{(1,15)} = 4.47, p < 0.001$), 4 μg/kg ($t_{(1,15)} = 5.35, p < 0.001$), and 8 μg/kg ($t_{(1,15)} = 9.27, p < 0.001$) doses of CCK significantly reduced intake within course one, whereas the 8 μg/kg ($t_{(1,15)} = 4.21, p = 0.001$) dose of CCK was the only dose that significantly decreased intake in course two. The 2 μg/kg ($t_{(1,15)} = 0.24, p = 0.811$), 4 μg/kg ($t_{(1,15)} = 1.71, p = 0.108$), and 8 μg/kg ($t_{(1,15)} = 2.37, p = 0.032$) doses of CCK did not significantly reduced intake within course three.
The meal type × course × injection interaction was significant ($F_{(12,180)} = 3.36, p = 0.007$; Figure 34a, b, c). A similar intake pattern was observed in the SC-SC-SC and SC-PB-MC meals. In both of these meals food intake within each injection condition tended to decrease as the courses progressed. However, in the MC-PB-SC meal, a different pattern was observed at all CCK doses, with intake increasing from the first to the second course, and then decreasing from the second to the third course.

**Discussion**

Dietary variety increased food intake by 27% when foods were presented in increasing order of preference, which is consistent with previous reports of dietary variety presented in a successive manner increasing food intake by 23% (Clifton et al., 1987) and 26% (Rolls, B.J. et al., 1982). In contrast, dietary variety presented in decreasing order of preference did not significantly increase food intake. These results suggest that the intake-enhancing effects of dietary variety can be influenced by the manner in which foods are presented. Whether an animal comes into contact with foods in increasing order of preference or decreasing order of preference affects the total amount of food consumed. Specifically, coming into contact with the foods in an increasing order of preference increases food intake to a greater extent than when foods are experienced in the opposite order.

The animal's preference for the various foods that are presented successively appears to affect the pattern of consumption as well. When food is experienced in a decreasing order of preference or the same food is experienced in a successive fashion, food intake tends to decrease as each course is experienced. The similar pattern observed between these two very different meal
types may be explained in terms of changes in palatability. Because the foods used in these experiments are all similar in caloric value and have similar amounts of fat it is likely that animals' selections of foods are predominately related to the palatability of the foods rather than to other factors. In the single-food meal where the same food is available for consumption during each course, the palatability of the food will tend to decrease as the meal progresses, and food intake will decline from one course to the next. Likewise, when several foods are presented successively, and in decreasing order of preference, palatability will also decrease as the meal progresses, and food intake will decline from one course to the next.

On the other hand, if foods are encountered in an increasing order of preference, the intakes in the various courses may be more similar, and there may even be increases in intake from one course to the next if the difference in preference from one course to the next is great enough. Therefore, presenting foods in an increasing order of preference means that foods are also being presented in an increasing order of palatability. It has been postulated that when a single food is available for consumption eating decreases as time passes due to a decrease in palatability of the food (Rolls, B.J., Rolls et al., 1981; Rolls, B.J. et al., 1984). However, when foods in a multiple-food meal are available in an increasing order of palatability, it is less likely that eating will be decreased with the passage of time, because this reduction in palatability with time is no longer present to cause this slowing of food consumption. For example, in the MC-PB-SC meal (increasing order of preference), intake increased from the first course to the second course, and intakes in the second and third course were relatively similar. Furthermore, the trends observed in both the SC-PB-MC meal and the MC-PB-SC meal tend to be consistent regardless of the dose of
CCK the animal is injected with. Thus, it appears that preference can have a substantial impact on the patterns of food consumption and on the effectiveness of dietary variety to increase food intake.

In Experiment 4, CCK was again found to inhibit food intake in a dose-dependent manner, with 2 - 8 μg/kg doses of CCK inhibiting food intake between 20 and 48%, which is consistent with previous findings that 1-16 μg/kg doses of CCK inhibited food intake from 29 to 71% (Moran et al., 1998). Also, the inhibitory effects of CCK on food intake appear to be similar regardless of meal type (single-food and multiple-food). This is consistent with the finding of Experiment 3, that CCK inhibited food intake to a similar extent in single-food and multiple-food (simultaneous presentation) meals. Therefore, CCK is equally effective at reducing food intake when foods are presented either simultaneously or successively.

In the previous experiments it has been repeatedly demonstrated that the administration of exogenous CCK inhibits food intake. However, these experiments do not provide any insight into the mechanism of action of exogenous CCK. Therefore, Experiment 5 directly examines the role of CCK_A receptors in the mediation of the effects of exogenous CCK through the use of a CCK_A antagonist.

Experiment 5

Experiments 1-4 did not examine the location of action of exogenous CCK. Accordingly, Experiment 5 was conducted to demonstrate that CCK reduced food intake by stimulating CCK_A receptors.
Lorglumide, which specifically antagonizes CCK₄ receptor types (Greenberg, Torres, Smith, & Gibbs, 1989), has been shown to reverse the inhibitory effects of exogenous CCK-8 on food intake. For example, Schneider, Murphy and Smith (1988) injected 18-hr food-deprived rats with lorglumide 15 min prior to a 30-min test meal. They found that 200 and 2000 μg/kg doses of lorglumide significantly antagonized the inhibitory effects on food intake of a 8 μg/kg dose of CCK by 74% and 82%, respectively. In other research, 17-hr food deprived rats were injected with lorglumide 30 min and with CCK-8 immediately prior to a 30-min test meal of cookies. It was reported that a dose range of 50 - 100 μg/kg of lorglumide partially antagonized the effects of a 5 μg/kg dose of CCK on food intake by approximately 50% (Makovec, Bani, Chiste, Revel, Rovati, & Sentnikar, 1986).

Lorglumide has been demonstrated to also reverse the inhibitory effects of CCK-8 on sham feeding. For example, Yox, Brenner, & Ritter (1992) administered a 600 μg/kg dose of lorglumide to 15-hr to 19-hr food-deprived rats 10 min prior to a 30-min sham-feeding test. They found that this dose of lorglumide significantly antagonized the suppressive effects of a 2 μg/kg dose of CCK by 78%. Therefore, lorglumide appears to be quite effective at antagonizing the inhibitory effects of CCK on food intake.

In Experiment 5, rats received two injections prior to the presentation of either a 30-min single-food or multiple-food meal. Rats were injected with either sodium bicarbonate or lorglumide 10 min prior to the test meal, and then with either saline or CCK-8 5 min prior to the meal. Lorglumide was selected as the CCK₄ antagonist for two reasons. First, it is a highly specific CCK₄ antagonist that has previously been shown to antagonize the inhibitory effects of
exogenous CCK. In addition, the effects of lorglumide have never been examined in non-deprived animals. A 600 μg/kg dose of lorglumide was selected because in previous research it was demonstrated that the 600 μg/kg dose substantially antagonized the effects of CCK on sham feeding in food-deprived rats (Yox et al., 1992). Furthermore, this dose falls between the 200 and 2000 μg/kg doses of lorglumide that were shown to antagonize the effects of an 8 μg/kg dose of CCK by up to 74% and 82%, respectively (Schneider, Murphy, & Smith, 1988). Therefore, the 600 μg/kg dose of lorglumide should be large enough to substantially influence the effects of CCK, but not so large as to potentially cause aversive effects. The goal was to determine whether lorglumide would antagonize the inhibitory effects of peripherally administered exogenous CCK on the intake of single-food and multiple-food meals.

The hypotheses for Experiment 5 were as follows:

**Hypothesis 5.1.** When the foods in the multiple-food meal are presented simultaneously intake in the multiple-food meals will be greater than intake in the single-food meal.

**Hypothesis 5.2.** Administration of exogenous CCK-8 will cause a decrease in food consumption.

**Hypothesis 5.3.** Lorglumide will significantly antagonize the inhibitory effects of peripherally administered CCK on food intake.

**Methods**

**Subjects and Housing**

In Experiment 5, 16 naive male Wistar rats were used. At the start of the experiment they
weighed 330 - 410 g. The housing conditions were the same as in the previous experiments.

Materials

The foods (MC, PB, & SC) were the same as those utilized in the previous experiments. In this experiment, only the 8 μg/kg dose of CCK was used. The CCK solution had a concentration of 4 μg/ml, and it was injected in a volume of 2 ml/kg. The CCK vehicle, 0.15M saline, was administered in a volume of 2 ml/kg. Lorglumide (ICN Canada, Montreal PQ) was dissolved in 1% sodium bicarbonate (Na$_2$CO$_3$) by gentle agitation and the pH readjusted to 7.4 with glacial acetic acid (CH$_3$COOH), as described by Schneider et al. (1988). A solution with a concentration of 300 μg/ml lorglumide was prepared and injected i.p. in a volume of 2 ml/kg, resulting in a dose of 600 μg/kg. The antagonist vehicle was 1% sodium bicarbonate, administered in a volume of 2 ml/kg.

Procedure

Before the study commenced, the rats were pre-exposed to each of the three foods for one 24-hour session and one 30-minute session. Furthermore, starting three days prior to the commencement of Experiment 5, rats received a mock injection on each of two consecutive days. The rats were tested under eight conditions, with each of two meal types (single-food and multiple-food) paired once with each of four injection pairs (saline-Na$_2$CO$_3$, saline-lorglumide, CCK-Na$_2$CO$_3$, and CCK-lorglumide). The two types of meal used were a multiple-food meal consisting of 28g of each of the three foods presented simultaneously, and a single-food meal consisting of approximately 28g of PB. PB was used in the single-food meals because in previous experiments its intake was consistently between that of MC and SC. The use of PB therefore
would permit easier detection of both increases and decreases in consumption than would either MC or SC, because of potential floor effects with MC and potential ceiling effects with SC. The general procedures were the same as in previous experiments. Rats were first injected i.p. with either lorglumide (600 μg/kg) or 1% sodium bicarbonate 10 minutes before each 30-min test meal, and then they were injected i.p. with CCK (8 μg/kg) or 0.15M saline 5 minutes later. Rats were weighed on each day of testing. Testing occurred every second day over a sixteen day period, between 4:30 and 7:00 pm.

**Results**

**Food Intake in the Single-food and Multiple-food Meals**

The data from one rat were not included in the analysis because this rat consumed less than 0.02 g of PB on all eight days of testing. The data from the remaining 15 rats were analyzed using a $2 \times 2 \times 2$ repeated-measures ANOVA, with two levels each of lorglumide dose (0 & 600 μg/kg), CCK dose (0 & 8 μg/kg), and meal type (single-food and multiple-food). There were significant main effects of meal type ($F_{(1,14)} = 17.64, p = 0.001$; Figure 35), with 41% more food being consumed in the multiple-food meal than in the single-food meal. There was a significant main effect of the CCK condition ($F_{(1,14)} = 22.64, p < 0.001$; Figure 36), with CCK inhibiting food intake by 32%. Also, there was a significant main effect of the lorglumide condition ($F_{(3,45)} = 21.94, p < 0.001$; Figure 37), with intake being 26% greater in the lorglumide condition than in the sodium bicarbonate condition. However, the lorglumide and sodium bicarbonate conditions were averaged across the CCK and saline conditions, so the main effect of lorglumide does not provide a true reflection of the effect of only lorglumide on food intake.
Of greatest interest was the finding of a significant CCK × lorglumide interaction effect ($F_{(1,14)} = 14.76, p = 0.002$; Figure 38). CCK significantly inhibited food intake by 46% when preceded by sodium bicarbonate ($t_{(1,14)} = 6.70, p < 0.001$), but by only 18% when preceded by lorglumide (not significant; $t_{(1,14)} = 2.27, p = 0.040$; note $\alpha = 0.016$). Thus, lorglumide offset the effect of CCK by 62%. Furthermore, food intake was not significantly different between the sodium bicarbonate-saline and the lorglumide-CCK conditions ($t_{(1,14)} = 1.78, p = 0.097$; note $\alpha = 0.016$). The sodium bicarbonate-saline condition provides a baseline measure of intake as the animals are receiving injections of the two vehicle solutions. Therefore, since food intake in the lorglumide-CCK condition was not significantly different from what could be considered baseline intake, this suggests that lorglumide almost completely reversed the inhibitory effects of CCK on food intake. In addition, food intake was not significantly different between the lorglumide-saline and sodium bicarbonate-saline conditions ($t_{(1,14)} = -1.26, p = 0.229$; note $\alpha = 0.016$), indicating that lorglumide by itself did not cause any significant change in food intake.

The CCK × meal type interaction was also significant ($F_{(1,14)} = 25.72, p = 0.027$; Figure 39). CCK significantly inhibited food intake by 37% in the multiple-food meal ($t_{(1,14)} = 5.02, p < 0.001$), but by only 24% in the single-food meal ($t_{(1,14)} = 2.44, p = 0.028$; note $\alpha = 0.025$).

The meal type × lorglumide interaction was not significant ($F_{(1,14)} = 1.28, p = 0.278$; Figure 40). Therefore, it appears that lorglumide has a similar effect on food intake in single-food and multiple-food meals. Finally, the CCK × meal type × lorglumide interaction was also not significant ($F_{(1,14)} = 0.97, p = 0.342$; Figures 41a, b).
Discussion

As in previous experiments, dietary variety increased food intake and CCK inhibited food intake. As mentioned in Experiments 1 - 4, these findings are consistent with a number of previous reports which show that providing multiple-food as opposed to a single-food meals causes greater food intake (Clifton et al., 1987; Rolls, B.J. et al., 1982), and that injecting rats with exogenous CCK causes a decrease in food intake (Moran et al., 1998).

Furthermore, it was found that CCK was less effective at reducing food intake in single-food meals than in multiple-food meals. This finding is opposite to the prediction that follows from the recent-foods hypothesis and is more consistent with the all-foods hypothesis, which suggests that CCK reduces the palatability of foods in general, and not just the palatability of recently consumed foods. Furthermore, these results may also suggest that the acceptability of the food may be an important factor when determining the inhibitory effectiveness of CCK. Thus, it could be the case that meal type is not the important factor, but rather the acceptability of the food. For example, it is possible that CCK may be less effective at reducing the intake of a multiple-food meal consisting of foods that are not readily accepted (or that are unpalatable) than a single-food meal consisting of a very readily accepted food (or that is highly palatable).

In addition, the 600 µg/kg dose of lorglumide antagonized the inhibitory effects of peripherally administered CCK (8 µg/kg) by 62%, which is consistent with previous reports that 200 - 2000 µg/kg doses of lorglumide antagonized the effects of a 8 µg/kg dose of CCK by 62% - 82% (Schneider, Murphy, & Smith, 1988). This finding is also consistent with the report that a
600 μg/kg dose of lorglumide reversed the inhibitory effects of a 2 μg/kg dose of CCK on sham feeding by 78%.

The finding that lorglumide reverses the inhibitory effects of exogenous CCK is also consistent with previous findings that other CCK<sub>A</sub>-receptor antagonists reverse the inhibitory effects of exogenous CCK on food intake. For example, the CCK<sub>A</sub>-antagonist devazepide administered to rats in doses of 100 and 300 μg/kg completely reverses the inhibitory effects of a 8 nmol/kg dose of CCK (Reidelberger & O’Rourke, 1989). Moran et al. (1992) also demonstrated that devazepide antagonizes the intake-reducing effects of CCK in rats, with 32 and 100 μg/kg dose of devazepide completely reversing the effects of a 4 μg/kg dose of CCK. As well, in mice it was demonstrated that a 100 μg/kg dose of devazepide completely reverses the inhibitory effects of a 10 μg/kg dose of CCK. Thus, these results provide further support that exogenous CCK inhibits food intake predominantly by acting on CCK<sub>A</sub> receptors.

Furthermore, it was also determined that lorglumide antagonized the effects of CCK to a similar extent in single-food and multiple-food meals. This suggests that CCK has the same mode of action in both meal types, that is, CCK inhibits food intake in both types of meals by acting on CCK<sub>A</sub> receptors.

Although it has been repeatedly demonstrated that CCK<sub>A</sub> antagonists reverse the inhibitory effects of exogenous CCK, the results have not been so clear with respect to the effects of these antagonists on the inhibitory effects of endogenous CCK. Studies examining whether CCK<sub>A</sub> antagonists can reverse the effects of peripheral endogenous CCK thereby causing an increase in food intake, have produced conflicting results. For example, there are some reports that where
devazepide is administered alone rather than in conjunction with CCK, it can increase food intake. However, other researchers have not found this to be the case. In one report, 300 and 1000 μg/kg doses of devazepide administered to 1-hr food-deprived rats did not significantly increase solid food intake after 2 hr of testing, but these two doses did significantly increase intake by 20% and 23%, respectively, after 3 hr of testing and by 13% and 22%, respectively, after 21 hr of testing (Reidelberger & O’Rourke, 1989). Moran et al. (1992) provide further support that devazepide can reverse the inhibitory effects of endogenous CCK. They reported that in 6-hr food-deprived rats, 32 and 100 μg/kg doses of devazepide significantly increased the intake of a glucose solution at 15, 30, 45, and 60 min into a 60 min feeding test, with intake being increased between approximately 37% and 44%. On the other hand, a number of researchers have reported that devazepide in varying doses does not increase food intake (as reviewed by Schneider et al., 1988).

In addition, several studies have shown that lorglumide does not appear to increase food intake when injected prior to food intake in fasted rats. For example, a dose range of 10 - 10000 μg/kg of lorglumide administered i.p. 30 minutes prior to intracerebroventricular administration of saline did not significantly increase 30-minute food intake in seventeen-hour fasted rats (Makovec et al., 1986). In other studies, i.p. injections of 300 and 600 μg/kg doses of lorglumide did not significantly increase food intake in 15 to 19-hr food deprived rats (Greenberg et al., 1989; Yox et al., 1992). However, all the above mentioned research on lorglumide involved fasted rats. Therefore, it is possible that lorglumide did not increase food intake in the above reports because the rats had been fasted between 15-19 hr. This extreme degree of deprivation may have caused rats to eat such large quantities of food that any excitatory effects of lorglumide were overridden
by other factors that normally act to terminate meals, such as distention of the gastrointestinal tract (Smith & Gibbs, 1998).

The purpose of Experiment 5 was to examine the role of CCK$_A$ receptors in the mediation of exogenous CCK and not to examine the role of endogenous CCK in the control of food intake. However, in Experiment 5 it was found that lorglumide did not increase food intake in either single-food or multiple-food meals when administered to saline-injected rats. The absence of a significant increase in food intake is consistent with the above mentioned finding that lorglumide does not increase food intake when administered by itself (Makovec et al., 1986; Yox et al., 1992; Greenberg et al., 1989). These results may suggest that either endogenous CCK is not present in a high enough quantity in the periphery to affect food intake or that the experimental paradigm is not effective at detecting the effects of lorglumide on food intake. For example, the effects of lorglumide on food intake may take longer than 30 min to be manifested. If so, then extending the testing time may allow for the detection of any effects that lorglumide may have on food intake. Accordingly, Experiment 6 was conducted to examine the effects of lorglumide on food intake over an extended period of time to more directly examine the role of endogenous CCK in the control of food intake.

Experiment 6

As discussed, the previous experiments provide little information about the effects of endogenous CCK on food intake. However, Experiment 5 indicates that blocking endogenous CCK has no effect on 30-min food intake. The purpose of Experiment 6 was to examine the
effects of endogenous CCK by administering a CCK\textsubscript{A} antagonist alone prior to single-food and multiple-food meals lasting several hours.

If CCK reduces food intake by reducing the palatability of foods, then blocking the effects of endogenous CCK should lead to less of a reduction in the palatability of foods with continued intake than if its effects were present. Therefore, administration of a CCK\textsubscript{A} antagonist should lead to an increase in food intake. Furthermore, because there are a number of foods available in a multiple-food meal compared to a single-food meal, intake would most likely be stimulated to a greater extent in a multiple-food meal than a single-food meal.

In Experiment 6, non-food deprived rats were injected with the CCK\textsubscript{A}-antagonist lorglumide 10 min prior to the presentation of either a 6-hr single-food or multiple-food meal. Non-deprived rats were used in Experiment 6 for two reasons. First, as in Experiments 1-5, non-deprived rats were used in Experiment 6 because the effects of dietary variety are more likely to be observed in non-deprived than deprived rats (Rolls, B.J. et al., 1983; Treit et al., 1983). In addition, in past research lorglumide has not been found to increase food intake in deprived animals (Makovec et al., 1986; Yox et al., 1992; Greenberg et al., 1989).

Although feeding tests were only 30 min long in Experiments 1-5, food intake was measured for 6 hr in Experiment 6 because the 30-min testing period in Experiment 5 may have been too short to detect the effects of lorglumide. Only one of the previously published studies has examined the effects of lorglumide on food intake in a feeding test that lasted longer than 30 minutes. Greenberg et al. (1989) found that i.p. injection of a 600 μg/kg dose of lorglumide did not increase sham feeding during a 90-min period in 17-hr food-deprived rats. Moreover, in one
study examining the effects of devazepide on food intake, devazepide did not significantly increase solid food intake at + 2 hr after the presentation of the test meal, but did significantly increase intake by + 3 hr (Reidelberger & O’Rourke, 1989). Therefore, the effects of lorglumide on food intake may be more easily detected in a longer test session.

The hypotheses for Experiment 6 were as follows:

**Hypothesis 6.1.** When the foods in a multiple-food meal are presented simultaneously intake in multiple-food meals will be greater than intake in single-food meal.

**Hypothesis 6.2.** Lorglumide will increase food intake when administered i.p. as compared to intake when the vehicle is injected i.p.

**Hypothesis 6.3.** Lorglumide will increase intake more in multiple-food meals than in single-food meals.

### Methods

**Subjects and Housing**

In this experiment the 16 male Wistar rats used in Experiment 5 served as subjects. At the start of the experiment they weighed approximately 390 - 500 g. The housing conditions were the same as in the previous experiments.

**Materials**

The same foods (MC, PB, & SC) were used as in the previous experiments. The same concentration of lorglumide (300 µg/ml) was used as in Experiment 5 and administered i.p. in the same volume to yield a dose of 600 µg/kg.
Procedure

The rats were tested under four conditions, with each of two meal types (single-food and multiple-food) paired with each of two injection conditions (0 & 600 μg/kg doses of lorglumide). The procedure utilized was generally similar to that of Experiment 5. Again, PB was used as the single-food meal. However, rats received a single injection of either lorglumide or sodium bicarbonate which was administered 10 min prior to the test meal. A second difference in procedure was that the test meal was 6 hr rather than 30 min in duration, and food intake was recorded at +2, +4, and +6 hr. Testing took place every second day over an eight day period, between 1:00 and 8:30 pm.

Results

Food Intake in the Single-food and Multiple-food Meals

The data were analyzed using a $2 \times 2 \times 3$ repeated-measures ANOVA, with two meal types (single- vs multiple-food), two levels of the injection condition (0 & 600 μg/kg doses of lorglumide), and three time periods (each two hours in length). This analysis revealed that there was a significant main effect of meal type ($F_{(1,14)} = 10.15, p = 0.007; \text{Figure 42}$), with 28% more food consumed in the multiple-food meal than in the single-food meal. Also, there was a significant main effect of time ($F_{(2,28)} = 80.51, p < 0.001; \text{Figure 43}$), with food intake decreasing over time. There was no significant main effect of the injection condition ($F_{(1,14)} = 0.671, p = 0.119; \text{Figure 44}$). Thus, lorglumide did not significantly increase food intake.

None of the following interactions proved significant: meal type × time ($F_{(2,28)} = 1.28, p = 0.294; \text{Figure 45}$), injection × time ($F_{(2,28)} = 2.00, p = 0.171; \text{Figure 46}$), meal type × injection
(F_{1,14} = 1.06, p = 0.320; Figure 47), and meal × injection × time (F_{2,28} = 0.39, p = 0.637; Figure 48a, b). Therefore, the 600 μ/kg dose of lorglumide did not significantly increase intake in either of the meal types during any of the time periods.

**Discussion**

Food intake was 28% greater in the multiple-food meal than in the single-food meal, providing further support that dietary variety can increase food intake. Again, this is consistent with a number of findings that dietary variety enhances food consumption between 23% - 33% (Clifton et al., 1987; Rolls, B.J. et al., 1982; Treit et al., 1983).

Furthermore, even with a lengthened testing period lorglumide did not increase food intake, which is consistent with previous reports that lorglumide does not increase food intake (Greenberg et al., 1989; Makovec et al., 1986; Yox et al., 1992). It is not likely that lorglumide’s failure to increase food intake was the result of a ceiling effect, because dietary variety did increase food intake. If there was a ceiling effect one might expect that lorglumide would not have increased the intake of the multiple-food meal, but it certainly could have increased the intake of PB, the food presented in the single-food meal.

The reason for lorglumide’s failure to increase food intake is unclear. However, it is possible that food intake was not increased because peripheral endogenous CCK may play only a relatively minor role in the control of food intake. Indeed, research using CCK$_A$ antagonists has not generally provided support for a major role for peripheral endogenous in the control of food intake. For example, as mentioned previously, research with the CCK$_A$-antagonist devazepide has produced mixed results, with positive results being reported most often when high doses are used
One group of researchers has reported increased food intake of up to 50% or more in response to devazepide administration (Hewson, Leighton, Hill, & Hughes, 1988). However, some studies have failed to demonstrate significant increases in food intake (Khosla, & Crawley, 1988; as reviewed by Schneider, Murphy, Gibbs, & Smith, 1988), and in other instances the reported increases have been quite small (<15%; Dourish, Coughlan, Hawley, Clark, & Iversen, 1988; as reviewed by Schneider, Murphy, Gibbs, & Smith, 1988). Furthermore, on a number of occasions, positive effects have only been found in response to a single dose of devazepide that lies in the middle of a range of doses that were used (as reviewed by Schneider, Murphy, Gibbs, & Smith, 1988).

It has also been demonstrated that other CCK$_A$-antagonists, such as 2-NAP (Ebenezer & Baldwin, 1995) and proglumide (Collins, Walker, Forsyth, and Belbeck, 1983; Schneider, Gibbs, & Smith, 1986), do not reliably increase food intake. Although there have been a few reports of proglumide increasing food intake (Shillabeer & Davison, 1984), others have failed to replicate this finding (Schneider et al., 1986). Thus, it still remains unclear to what extent endogenous CCK plays a role in the control of food intake.

**General Discussion**

Previous research has demonstrated that dietary variety increases food intake in rats by 23% - 33% (Clifton et al., 1987; Rolls, B.J. et al., 1983; Treit et al., 1983). In the six experiments conducted, dietary variety increased food intake by 18% to 133% providing further support that dietary variety may cause increased food intake. Increased food intake was found when dietary
variety was presented both simultaneously (Experiments 1, 2, 3, 5, and 6) and successively (Experiment 4). However, successive presentation of a variety of foods was found to enhance intake only when foods were presented in an increasing order of preference.

In addition, it has been demonstrated in previous research that there is a clear dose-response relationship between CCK and food intake. For example, 2 - 8 μg/kg doses of CCK reduced intake between 34% - 54% in one report (Moran et al., 1997) and between 43% - 67% in another (Moran et al., 1998). It was consistently found in the experiments reported here that CCK reduced food intake in a dose-dependent fashion, with 2 - 8 μg/kg doses of CCK reducing food intake between 20% - 48%. These results provide further support for the notion that exogenous CCK causes a reduction in food intake.

Of particular interest is the finding that CCK reduced food intake to a similar extent in both single-food and multiple-food meals, regardless of whether the foods within the multiple-food meal were presented simultaneously or successively. Therefore, the inhibitory effectiveness of exogenous CCK does not appear to be affected by dietary variety. Furthermore, these results suggest that to the extent that CCK reduces food intake by reducing the palatability of food, it does so by reducing the palatability of all food, as opposed to reducing the palatability only of those foods that have recently been consumed. Future research might address the effectiveness of centrally administered CCK in the context of single-food and multiple-food meals.

Although the inhibitory effectiveness of CCK is not affected by dietary variety, it may be affected by the palatability of the available food. When foods are not readily consumed, as was the case for MC in the experiments reported here, CCK is much less effective at reducing intake.
Therefore, this may suggest that a certain amount of food needs to be consumed before CCK will inhibit food intake. Furthermore, if CCK reduces food intake by reducing the palatability of foods, then intake of highly palatable foods may be more affected by CCK than intake of less palatable foods. If a food is not very palatable to begin with then reducing its palatability will not have as much of an effect on its intake, but if a food is highly palatable then reducing its palatability may have a very large impact on the amount that is consumed. Thus, these results suggest that palatability may play a substantial role in mediating the effects of CCK. Further research is needed to verify this conclusion. For example, a follow-up study to the Waldbillig and Bartness (1982) research on the effects of CCK on different concentrations of sucrose solutions could be conducted. Waldbillig and Bartness (1982) injected rats with CCK prior to presenting one of three different concentrations of sucrose solutions. They found that the intake-reducing effects of CCK were greater when more highly concentrated sucrose solutions were available for consumption. Based on this finding, Waldbillig and Bartness (1982) suggested that the effectiveness of CCK to reduce intake is a direct function of the palatability of the food being consumed; that is, as the palatability of food increases, CCK becomes increasingly effective at reducing the intake of the food. However, because the palatability of the solutions that were used was confounded with their caloric density, it cannot be determined whether CCK’s effectiveness is related to palatability or to caloric density. Of course, the palatability and the caloric density of the solutions could be separated by modifying the procedure of Waldbillig and Bartness (1982). For example, one concentration of sucrose solution could be used and the palatability of this solution could be manipulated by adding either a non-caloric sweetener (e.g. saccharin) to increase its palatability or
a sour tasting substance such as citric acid to reduce its palatability. Therefore, the three solutions would be isocaloric and identical in macronutrient content, but they would differ in their palatability. Rats would be injected with CCK prior to the individual presentation of each of these sucrose solution to better determine to what extent palatability affects the intake-reducing effects of CCK.

It has been previously demonstrated that the CCK_A-antagonist lorglumide antagonizes the inhibitory effects of exogenous CCK on food intake (Inui et al., 1987; Schneider, Murphy, & Smith, 1988), suggesting that CCK reduces food intake by acting on CCK_A receptors. Therefore, the finding in Experiment 5 that lorglumide antagonizes the effects of peripherally administered exogenous CCK provides further evidence that exogenous CCK reduces food intake by activating CCK_A receptors. In addition, lorglumide antagonized the intake-reducing effects of CCK to a similar extent in both single-food and multiple-food meals suggesting that exogenous CCK reduces the intake of both types of meal by the same mechanism of action, i.e. through the activation of CCK_A receptors.

Although lorglumide and other CCK_A antagonists antagonize the effects of exogenous CCK, research using CCK_A antagonists has not provided clear evidence for a role for endogenous peripheral CCK in the control of food intake. For example, in previous research, lorglumide by itself has not been found to cause an increase in food intake (Greenberg et al., 1989; Makovec et al., 1986; Yox et al., 1992). As previously mentioned, the effects of other CCK_A antagonists on food intake are also unclear. For example, some researchers have found that devazepide increases food intake, whereas other have been unable to replicate this finding (as reviewed by Schneider,
Murphy, Gibbs, & Smith 1988). In Experiments 5 and 6, lorglumide did not cause increased food intake when administered by itself prior to food presentation. Taken together, these predominately negative findings suggest that peripheral endogenous CCK may not play a substantive role in the control of food intake under normal circumstances. However, there may be alternative explanations for these negative findings, as there could be unknown factors that affect the ability of antagonists to block the effects of endogenous CCK. This may explain why there are such mixed findings with regards to the ability of CCK\textsubscript{A} antagonists to increase food intake (as reviewed by Schneider, Murphy, Gibbs, & Smith, 1988). There might have been a greater likelihood of observing an increase in food intake if the CCK\textsubscript{A}-antagonist devazepide had been used in the experiments reported here, given that devazepide has sometimes been found to increase food intake, i.e., to antagonize the inhibitory effects of endogenous CCK (Reidelberger & O’Rourke, 1989; Silver et al, 1989; Strohmayer & Greenberg, 1996). Future research examining the effects of other CCK antagonists on food intake could provide valuable insights into the role of endogenous CCK in the control of food intake, and they may yet reveal differential effects on total food intake in single-food and multiple-food meals. For example, the CCK\textsubscript{A}-antagonist 2-NAP, which is thought to not cross the blood-brain barrier (Ebenezer & Baldwin, 1995), would permit a more specific examination of the effects of peripheral endogenous CCK on food intake. It should be noted that central processes may also be involved in the control of food intake by CCK, but these mechanisms are outside of the focus of this thesis.

The literature reveals that there are a number of factors that can affect food intake when dietary variety is available, such as the extent of the variety available in the multiple-food meal
(Roll, B.J. et al., 1981; Rolls, B.J. et al., 1982), individual differences (Speigel & Stellar, 1990; Pliner, Polivy, Zakalusny, 1980; Rolls, B.J. et al., 1991), food deprivation (Treit et al., 1983), stress (Zylan & Brown, 1996), type of foods presented during a meal (Vandewater & Vickers, 1996), and temporal factors (Meiselman et al., 2000; Rolls, B.J. & De Waal, 1985). In the present research, it was found that preference can also affect the magnitude of the variety effect. In Experiment 4, dietary variety increased food intake when foods were presented in increasing order of preference, whereas dietary variety did not significantly increase intake when foods were presented in a decreasing order of preference. The pattern of intake was similar in the single-food meal and in the multiple-food meal in which foods were presented in decreasing order of preference. Specifically, in both types of meal, intake decreased with each consecutive course. This pattern was most likely evident because in both of these meals palatability decreased with each course. Because the palatability of the food(s) decreased with continued consumption in both meals, this led to a decrease in intake with each course as well. On the other hand, in the multiple-food meal in which foods were presented in increasing order of preference, food intake was relatively constant in each course, or even increased from one course to the next. This pattern was likely observed because the foods increased in palatability with each course, whereas the opposite was true for the other two types of meals.
References


Dourish, C.T., Coughlan, J., Hawley, D., Clark, M., Iversen, S.D. (1988). Blockade of CCK-induced hypophagia and prevention of morphine tolerance by the CCK antagonist, L364,


Waldbillig, R.J., & Bartness, T.J. (1982). The suppression of sucrose intake by cholecystokinin is scaled according to the magnitude of the orosensory control. *Physiology & Behavior, 28*, 591-595.


Table 1

Number of Test Meals in Experiments 1 and 2 in Which Rats Consumed the Entire Amount of at Least One of the Food(s) that Was/Were Available.

<table>
<thead>
<tr>
<th></th>
<th>MC</th>
<th>PB</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-food meals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 μg/kg CCK</td>
<td>0/11</td>
<td>1/11</td>
<td>5/11</td>
</tr>
<tr>
<td>8 μg/kg CCK</td>
<td>0/11</td>
<td>0/11</td>
<td>0/11</td>
</tr>
<tr>
<td>Multiple-food meals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 μg/kg CCK</td>
<td>0/11</td>
<td>0/11</td>
<td>1/11</td>
</tr>
<tr>
<td>8 μg/kg CCK</td>
<td>0/11</td>
<td>0/11</td>
<td>0/11</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-food meals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 μg/kg CCK</td>
<td>1/14</td>
<td>1/14</td>
<td>10/14</td>
</tr>
<tr>
<td>2 μg/kg CCK</td>
<td>1/14</td>
<td>1/14</td>
<td>1/14</td>
</tr>
<tr>
<td>4 μg/kg CCK</td>
<td>0/14</td>
<td>0/14</td>
<td>1/14</td>
</tr>
<tr>
<td>Multiple-food meals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0/14</td>
<td>2/14</td>
<td>4/14</td>
</tr>
<tr>
<td>2 μg/kg CCK</td>
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<td>1/14</td>
<td>0/14</td>
</tr>
<tr>
<td>4 μg/kg CCK</td>
<td>0/14</td>
<td>1/14</td>
<td>0/14</td>
</tr>
</tbody>
</table>
**Figure 1.** Experiment 1: Mean (+ SE) food intake in the multiple-food meal and the average of the single-food meals, averaged across injection condition.

*Significantly greater than the average of the single-food meals, F-test p < 0.050 (n= 11; p-value in this figure and all following figures are the cutoff values for significance and were calculated using the modified Bonferroni technique).
Figure 2. Experiment 1: Mean (+ SE) food intake in the saline and 8 ug/kg dose of CCK conditions, averaged across the average of the single food meals and the multiple-food meal. *Significantly greater than the 8 ug/kg dose, F-test p < 0.050 (n = 11).
Figure 3. Experiment 1: Mean (+ SE) food intake of the multiple-food meal and the average of the single-food meals within the saline and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.013 (n =11).
Figure 4. Experiment 1: Mean (+ SE) food intake in the multiple-food and single-food meals, averaged across injection condition. Values with the same indicators are significantly different, t-test $p < 0.025$ ($n = 11$).
Figure 5. Experiment 1: Mean (+ SE) food intake in the saline and 8 ug/kg dose of CCK conditions, averaged across the four meal types. *Significantly greater than the 8 ug/kg dose, F-test p < 0.050 (n = 11).
Figure 6. Experiment 1: Mean (+ SE) food intake of the multiple-food and single-food meals within the saline and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.013 (n = 11).
Figure 7. Experiment 1: Mean (+ SE) intake of the three foods within the multiple-food meal, averaged across injection condition. Values with the same indicators are significantly different, t-test p < 0.033 (n = 11).
Figure 8. Experiment 1: Mean (+ SE) food intake in the saline and 8 ug/kg dose of CCK conditions, averaged across the three foods within the multiple-food meal. *Significantly greater than the 8 ug/kg dose, F-test p < 0.050 (n = 11).
Figure 9. Experiment 1: Mean (+ SE) intake of the three foods within the multiple-food meal within the saline and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.017 (n = 11).
Figure 10. Experiment 2: Mean (+ SE) food intake in the multiple-food meal and the average of the single-food meals, averaged across injection condition. *Significantly greater than the average of the single-food meals, F-test p < 0.050 (n = 14).
Figure 11. Experiment 2: Mean (+ SE) food intake in the 0, 2, and 4 ug/kg dose of CCK conditions, averaged across the average of the single-food meals and the multiple-food meal. Values with the same indicators are significantly different, t-test p < 0.033 (n = 14).
Figure 12. Experiment 2: Mean (+ SE) food intake of the multiple-food meal and the average of the single-food meals within the 0, 2, and 4 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.008 (n = 14).
Figure 13. Experiment 2: Mean (+ SE) food intake in the multiple-food and single-food meals, averaged across injection condition. Values with the same indicators are significantly different, t-test $p < 0.025$ ($n = 14$).
Figure 14. Experiment 2: Mean (+ SE) food intake in the 0 (saline), 2, and 4 ug/kg dose of CCK conditions, averaged across the four meal types. Values with the same indicators are significantly different, t-test p < 0.033 (n = 14).
Figure 15. Experiment 2: Mean (+ SE) food intake of the multiple-food and single-food meals within the 0 (saline), 2, and 4 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.008 (n = 14).
Figure 16. Experiment 2: Mean (+ SE) intake of the three foods within the multiple-food meal, averaged across injection condition. Values with the same indicators are significantly different, t-test $p < 0.033$ ($n = 14$).
Figure 17. Experiment 2: Mean (+ SE) food intake in the 0, 2, and 4 ug/kg dose of CCK conditions, averaged across the three foods within the multiple-food meal. Values with the same indicators are significantly different, t-test p < 0.033 (n = 14).
Figure 18. Experiment 2: Mean (+ SE) intake of the three foods within the multiple-food meal within the 0, 2, and 4 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.011 (n = 14).
Figure 19. Experiment 3: Mean (+ SE) food intake in the multiple-food meal and the average of the single-food meals, averaged across injection condition. *Significantly different than the average of the single-food meals, F-test p < 0.050 (n = 16).
Figure 20. Experiment 3: Mean (+ SE) food intake in the 0, 2, 4, and 8 ug/kg dose of CCK conditions, averaged across the average of the single-food meals and the multiple-food meal. Values with the same indicators are significantly different, t-test p < 0.025 (n = 16).
Figure 21. Experiment 3: Mean (+ SE) food intake of the multiple-food meal and the average of the single-food meals within the 0, 2, 4, and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test $p < 0.013$ ($n = 16$).
Figure 22. Experiment 3: Mean (+ SE) food intake in the multiple-food and single-food meals, averaged across injection condition. Values with the same indicators are significantly different, t-test $p < 0.025$ ($n = 16$).
Figure 23. Experiment 3: Mean (+ SE) food intake in the 0, 2, 4, and 8 ug/kg dose of CCK conditions, averaged across the four meal types. Values with the same indicators are significantly different, t-test p < 0.025 (n = 16).
Figure 24. Experiment 3: Mean (+ SE) food intake of the multiple-food and single-food meals within the 0, 2, 4, and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.006 (n = 16). Note: c and j, t-test p = 0.007.
Figure 25. Experiment 3: Mean (+ SE) intake of the three foods within the multiple-food meal, averaged across injection condition. Values with the same indicators are significantly different, t-test $p < 0.033$ ($n = 16$).
Figure 26. Experiment 3: Mean (+ SE) food intake in the 0, 2, 4, and 8 ug/kg dose of CCK conditions, averaged across the three foods within the multiple-food meal. Values with the same indicators are significantly different, t-test $p < 0.025$ ($n = 16$).
Figure 27. Experiment 3: Mean (+ SE) intake of the three foods within the multiple-food meal within the 0, 2, 4, and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.008 (n = 16). Note: a and c, t-test p = 0.009.
Figure 28. Experiment 4: Mean (+ SE) food intake in the multiple-food and single-food meals, averaged across injection condition and course. Values with the same indicators are significantly different, t-test p < 0.033 (n = 16).
Figure 29. Experiment 4: Mean (+ SE) food intake in each course, averaged across meal type and injection condition. Values with the same indicators are significantly different, t-test $p < 0.033$ ($n = 16$).
Figure 30. Experiment 4: Mean (+ SE) food intake in the 0, 2, 4, and 8 ug/kg dose of CCK conditions, averaged across meal type and course. Values with the same indicators are significantly different, t-test p < 0.025 (n = 16).
Figure 31. Experiment 4: Mean (+ SE) food intake of the multiple-food and single-food meals within the 0, 2, 4, and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test \( p < 0.011 \) (\( n = 16 \)).
Figure 32. Experiment 4: Mean (+ SE) food intake of the single-food and multiple-food meals within each course. Values with the same indicators are significantly different, t-test $p < 0.011 (n = 16)$. 
Figure 33. Experiment 4: Mean (+ SE) food intake during each course within the 0, 2, 4, and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test $p < 0.008$ ($n = 16$).
Figure 34a. Experiment 4: Mean (+ SE) food intake of the SC-SC-SC meal within each course and within the 0, 2, 4, and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.008 (n = 16).
Figure 34b. Experiment 4: Mean (+ SE) food intake of the MC-PB-SC meal within each course and within the 0, 2, 4, and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test $p < 0.008$ (n = 16).
Figure 34c. Experiment 4: Mean (+ SE) food intake of the SC-PB-MC meal within each course and within the 0, 2, 4 and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.008 (n = 16).
Figure 35. Experiment 5: Mean (+ SE) food intake in the multiple-food and single-food meals, averaged across the CCK and lorglumide conditions. *Significantly greater than the single-food meal, F-test $p < 0.050$ ($n = 15$).
Figure 36. Experiment 5: Mean (+ SE) food intake in the 0 and 8 ug/kg dose of CCK conditions, averaged across meal type and lorglumide condition. *Significantly greater than the 8 ug/kg dose of CCK condition, F-test p < 0.050 (n = 15).
Figure 37. Experiment 5: Mean (+ SE) food intake in the lorglumide and sodium bicarbonate conditions, averaged across CCK condition and meal type. *Significantly greater than the sodium bicarbonate condition, F test p < 0.050 (n = 15).
Figure 38. Experiment 5: Mean (+ SE) food intake in the 0 and 8 ug/kg dose of CCK conditions within the lorglumide condition. Values with the same indicators are significantly different, t-test $p < 0.016$ ($n = 15$).
Figure 39. Experiment 5: Mean (+ SE) food intake of the multiple-food and single-food meals within in the 0 and 8 ug/kg dose of CCK conditions. Values with the same indicators are significantly different, t-test p < 0.025 (n = 15).
Figure 40. Experiment 5: Mean (+ SE) food intake of the multiple-food and single-food meals within the lorglumide and sodium bicarbonate conditions. Values with the same indicators are significantly different, t-test $p < 0.025$ ($n = 15$).
Figure 41a. Experiment 5: Mean (+ SE) food intake of the single-food meal (PB) within the 0 and 8 µg/kg dose of CCK conditions and within the lorglumide condition. Values with the same indicators are significantly different, t-test p < 0.017 (n = 15).
Figure 41b. Experiment 5: Mean (+ SE) food intake of the multiple-food meal within the 0 and 8 ug/kg CCK conditions and within the lorglumide condition. Values with the same indicators are significantly different, t-test p < 0.017 (n = 15).
Figure 42. Experiment 6: Mean (+ SE) food intake in the multiple-food and single-food meals, averaged across lorglumide condition. *Significantly greater than single-food meal, t-test p < 0.050 (n = 15).
Figure 43. Experiment 6: Mean (+ SE) food intake during each two hour time period, averaged across meal type and lorglumide condition. Values with the same indicators are significantly different, t-test p < 0.033 (n = 15).
Figure 44. Experiment 6: Mean (+ SE) total food intake in the lorglumide and sodium bicarbonate conditions, averaged across meal type (n = 15).
**Figure 45.** Experiment 6: Mean (+ SE) intake of the multiple-food and single-food meals during each time period, averaged across lorglumide condition. Values with the same indications are significantly different, t-test $p < 0.017$ (n = 15).
Figure 46. Experiment 6: Mean (+ SE) food intake in the lorglumide and sodium bicarbonate conditions within each time period, averaged across meal type. Values with the same indicators are significantly different, t-test $p < 0.017$ (n = 15).
Figure 47. Experiment 6: Mean (+ SE) total intake of the multiple-food and single-food meals within the lorgumide and sodium bicarbonate conditions. Values with the same indicators are significantly different, t-test $p < 0.025$ ($n = 15$).
Figure 48a. Experiment 6: Mean (+ SE) intake of the single-food meal (PB) during each time period within the sodium bicarbonate and lorglumide conditions. Values with the same indicators are significantly different, t-test $p < 0.017$ (n = 15).
Figure 48b. Experiment 6: Mean (+ SE) intake of the multiple-food meal during each time period within the sodium bicarbonate and lorglumide conditions. Values with the same indicators are significantly different, t-test $p < 0.017$ ($n = 15$).