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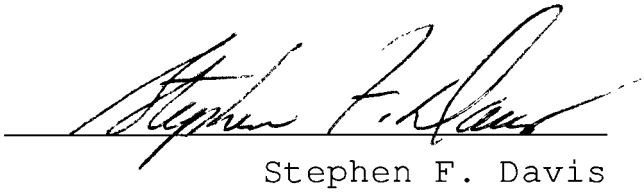
Abstract approved: 

Stephen F. Davis

A series of three experiments was performed to test the Rozin and Rodgers (1967) conditioned taste-aversion hypothesis of neophilia. All experiments involved the use of a protein- and tryptophan-deficient (grits) diet. In Experiment 1, deprived animals displayed preferences for a novel flavor. When given an alternative, grits raised subjects from Experiment 2 demonstrated aversions to a familiar fluid. The results from Experiment 3 indicate that the laboratory rat is capable of differentiating between two previously encountered flavors--one that was paired with normal laboratory chow, the other with the grits diet. The data from the three experiments extend the Rozin and Rodgers (1967) hypothesis using animals exposed to the experimental grits diet.

EXPOSURE TO A PROTEIN- AND TRYPTOPHAN-DEFICIENT DIET RESULTS IN NEOPHILIA

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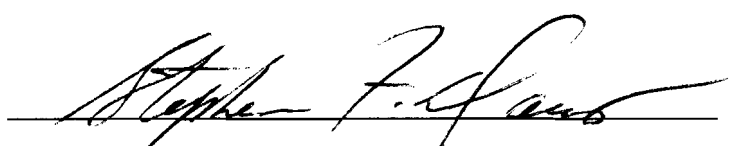

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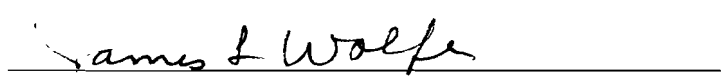

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CONTENTS

CHAPTER ONE

Introduction

The reaction of rodents to dietary stimuli, both novel and familiar, has been of interest in the psychology laboratory for many years (Luria, 1953; Richter, 1936; Richter, Holt & Barelare, 1937). Though researchers have maintained this interest and have struggled to gain further insight into the various influences of these stimuli upon ingestional behavior, much remains to be discovered. Before accurate predictions concerning responses to various food cues can be made, more information regarding response systems needs to be obtained. The present paper focused upon responses to an assortment of taste stimuli. The paper's application within the larger domain of taste-aversion learning, stemmed from the taste-aversion component of a theory first proposed by Rozin and Rodgers (1967), which will be addressed in the following pages.

A common paradigm for studying taste-aversion learning (Garcia, Hankins & Rusiniak, 1974; Kalat, 1974) involves: (a) Exposing experimental subjects to an unfamiliar taste stimulus (e.g., saccharin), (b) Subjecting them to some form of malaise or nausea, and

(c) Measuring the subjects' reaction to the same stimulus upon subsequent exposures. Provided adequate experimental control has been exercised, animals administered this routine will display stronger aversions (i.e. consume less of the novel taste stimulus) relative to control animals given a familiar water reinforcer during the conditioning episode. While taste-aversions have been formed between flavors and the effects of experimenter induced illnesses, such associations may also be formed between a particular food/fluid flavor and the physiological effects of that food or fluid.

The present series of experiments was conducted to examine some of the conditions under which laboratory animals will display preferences for unfamiliar tasting fluids. Taste neophilia is observed when an animal displays a preference for a new or novel tasting stimulus.

Its antonym, neophobia, is observed when an organism avoids a novel flavor stimulus. With respect to food/fluid stimuli, the neophobic response is commonly observed in animals that are given an alternative to an existing sufficient diet (Domjan; 1975,1977).

Novel food preferences in rats deficient in essential dietary components have been examined for several years (Harris, Clay, Hargreaves, & Ward, 1933; Richter et al., 1937; Rodgers & Rozin, 1966; Scott & Verney, 1947). For example, Rodgers and Rozin (1966) studied response patterns to new foods in animals raised on a diet deficient in thiamine (vitamin B1). The results clearly demonstrated that the thiamine-deficient rats, given an alternative to their familiar, insufficient diet, invariably preferred a novel food. On the other hand, if the new diet was thiamine-deficient and thiamine was added to the old diet, the animals returned to consumption of the more familiar foodstuff within three to four days (Rodgers & Rozin, 1966).

Through an examination of the effects of diets deficient in pyridoxine (vitamin B6) and riboflavin (vitamin B2), Rozin and Rodgers (1967) sought to extend the generality of their previous data. As predicted, rats deficient in these vitamins, when offered a choice of novel versus familiar foods, preferred the novel foods. Additionally, rats that had recovered from such deficiencies also preferred the novel diet when given the alternative.

Rozin (1976) attempted to delineate the mechanisms underlying the rat's ability to make adaptive choices from among a number of potential food alternatives and poisons. He reported that rats had a tendency to associate "new metabolic consequences of ingestion with new foods (p. 290)." When given a number of unfamiliar food choices, rats will sample sparingly from a single food item at a time, thus allowing for distinct evaluation of each individual substance. When consumption of a food source is followed by feelings of nausea or illness, the rat forms an aversion to that food and subsequently avoids it. Hence, when a rat is faced with a choice between a familiar food with which feelings of illness have been paired and some novel/unfamiliar food, it will select the new food.

Harper (1967) discussed the influence that the protein component of a diet can play in the regulation of food intake. It is suggested that plasma amino acid patterns are altered within two to four hours after consuming an imbalanced amino acid/protein food source (e.g., lacking an essential amino acid such as histidine) (Booth & Simson, 1971). The animal naturally associates the aversive consequences of having consumed an

imbalanced protein source with the appropriate food. In turn, this association triggers a response system that acts to decrease future intake of the particular food that had been sampled. Together these circumstances give a detailed account the naturally conditioned taste aversion discussed by Rozin and Rodgers (1966).

Another hypothesis, proposed to explain neophilic behavior is that animals learn specific hungers for each of a variety of dietary ingredients (Nachman & Cole, 1971; Richter, 1936). While attempting to procure these various ingredients, the animal comes into contact with both toxic and beneficial foods. By successfully selecting appropriate food sources, the laboratory rat has clearly demonstrated its ability to employ specific, adaptive behaviors (Rozin & Kalat, 1971).

To date, most of the research on the occurrence of neophilia has involved restrictions in dietary vitamins (Rozin & Rodgers, 1967; Seward & Greathouse, 1973). Thus, the effects of a variety of other essential dietary ingredients remain to be examined. One such ingredient is tryptophan. Tryptophan is an amino acid that is produced in the digestive system and is essential in

nutrition. It is apparently a precursor to the release of growth hormone (Becker, Davis, Grover, & Erickson, 1989), which in turn, causes living organisms to increase in physical size. Hence, a subnormal growth rate occurs in response to a dietary regimen insufficient in this protein building block. Namely, when compared to littermate controls raised on a nutritionally balanced diet, animals reared on a diet lacking in adequate protein and tryptophan are significantly smaller in size (Becker et al., 1989; Remley, Armstrong, Gilman, & Mercer, 1980).

The Rozin and Rodgers (1967) hypothesis would predict that animals exposed to a protein- and tryptophan-deficient diet would associate any adverse consequences with its flavor and subsequently form a taste-aversion to it. This taste-aversion would be exhibited when the animals were offered a choice between the familiar, aversive taste, and an unfamiliar, novel one (e.g., the rats would select the novel flavor).

The experiments to be reported were designed to evaluate the taste-aversion account of neophilia (Rozin and Rodgers, 1967). In all experiments the basic procedure involved

exposing animals to the protein- and tryptophan-deficient grits diet prior to preference tests that involved simultaneous exposure to both familiar and unfamiliar flavors. According to the taste-aversion account, animals exposed to the debilitating effects of the experimental diet should display a preference for a novel flavor (Experiment 1) and/or an aversion to a flavor that had been associated with the experimental diet (Experiments 2 and 3). The inclusion of groups of littermates raised on non-deficient diets allowed appropriate comparisons to be made between animals exposed to the experimental diet and those raised normally.

CHAPTER TWO

Experiment 1

Experiment 1 was conducted to determine whether neophilia would result following exposure to the protein- and tryptophan-deficient grits diet from weaning until 90-days-of-age. The neophilia hypothesis, proposed by Rodgers and Rozin (1967), would hold that any feelings of malaise or discomfort associated with the experimental diet would result in the formation of a taste-aversion to its particular flavor. Relative to control animal counterparts raised on a normal laboratory diet, animals exposed to the grits diet would be expected to prefer a novel taste over a familiar one when given a choice. By administering two consecutive preference tests to the animals, it was possible to examine both the occurrence of the neophilic effect and its duration.

Method

Subjects. Two litters of ten Holtzman-derived rat pups each, born and raised in the Emporia State University vivarium, served as subjects.

Apparatus. All testing took place in the home cage. Fluid consumption, calculated to the

nearest .50 ml, was measured using 50-ml polypropylene centrifuge tubes fitted with spill-resistant sippers.

Procedure. At birth the 2 litters were combined and the pups were randomly assigned to 1 of the 2 dams with which they remained until weaning at 21 days of age. The number of males and females within each group was equated as far as possible. At weaning, one-half of each litter was assigned to the experimental condition (Group Grits), and the other to the normal condition (Group Normal).

Upon being assigned to to its respective group, each animal was placed in a suspended wire-mesh cage and administered its designated diet on a free-feeding basis for the duration of the study. Group Grits animals received a mixture of Quaker instant grits and warm tap water, combined to a soupy consistency. Animals in the Normal diet condition received Purina Laboratory Chow on a free-feeding basis. Water was constantly available for subjects in both conditions. Weight data for each animal was obtained at 30, 60, and 90 days from birth.

At 90 days of age, all animals were placed on a fluid deprivation schedule which permitted them 15 minutes access to water daily. During

this three-day phase, baseline fluid consumption was recorded for each subject. Following this baseline consumption period, all animals were administered a 15-minute two-bottle preference test (peppermint versus water) for two consecutive days. The peppermint flavor was composed of 1.5% McCormick's Pure Peppermint extract in tap water.

Results

Due to the death of one experimental-diet (Grits) animal during the course of the experiment, unweighted means analysis of variance was used for all omnibus F tests. Analysis of the weight data yielded significance for the groups, $F(1,17) = 11.24$, $p < .01$, and days, $F(2,34) = 17.87$, $p < .01$, effects. Newman-Keuls post hoc analyses revealed that the Normal animals weighed significantly ($p < .01$) more than the Grits animals, and that the Normal animals gained a significant ($p < .01$) amount of weight between each respective weighing. These findings are supportive of the Becker et al. (1989, 1990) and Remley et al. (1980) reports demonstrating that animals exposed to the protein- and tryptophan-deficient grits diet weighed significantly less than their normal-diet counterparts.

Group comparisons were made via consumption ratios for each of the two preference testing days. These ratios were calculated by dividing the amount of peppermint consumed by the total amount of fluid (peppermint + water) consumed. Using this procedure, ratios higher than .50 mark peppermint preferences, while ratios lower than this amount denote a peppermint aversion.

A preliminary analysis of the fluid consumption data from preference testing failed to yield a significant gender effect. Hence, male and female subject data for each group were pooled. Analysis of this data yielded significant days, $F(1,17) = 6.75$, $p = .017$, and groups x days, $F(1,17) = 6.042$, $p = .023$, effects. Subsequent Newman-Keuls tests, used to examine the significant interaction, indicated that the consumption ratios of the Normal animals were significantly ($p < .05$) lower than those of the Grits animals on Day 1. By Day 2, however, the two groups did not differ reliably.

Figure 1 shows group mean consumption ratios for each of the two days of preference testing. All means were below .50, thus reflecting aversions to the novel peppermint flavor. Despite this finding, the higher group mean

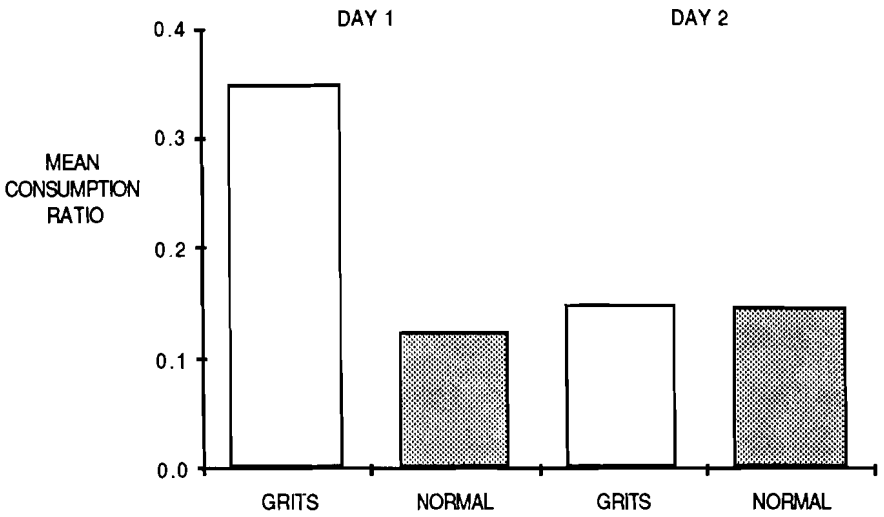

Figure 1. Group mean consumption ratios, calculated by dividing the amount of the target fluid (peppermint) consumed by the total amount of fluid consumed, for each of the 2 days of preference testing.

ratio for Group Grits, relative to that of Group Normal on Day 1, reflects the occurrence of neophilia.

Discussion

The results of this experiment clearly indicate that, relative to control-diet animals, animals maintained on the protein- and tryptophan-deficient grits diet will display the predicted preference for a novel flavor (i.e., they will display the neophilic effect). One

explanation is that the experimental animals had learned to associate the water from their diets with the "illness" resultant from the protein- and tryptophan-deprived diet. Due to this association, these animals were more willing to sample the unfamiliar-tasting peppermint solution.

Though the present data may lend some support to the Rozin and Rodgers (1967) hypothesis, the neophilic effects for rats from this experiment were rather short-lived. Stronger support could be given to the hypothesis if more lasting results were obtained.

CHAPTER THREE

Experiment 2

Experiment 1, a preliminary examination of the neophilic effect, produced results that lend support to the Rozin and Rodgers (1967) hypothesis. More specifically, protein- and tryptophan-deprived (Grits) rats consumed significantly more novel peppermint than did control-diet (Normal) subjects. According to the learned taste-aversion hypothesis, animals in the experimental group displayed a preference for the unfamiliar flavor due to the conditions set up when the familiar diet and its resulting illness and/or discomfort became associated.

However, while those results do indicate a preference for the new taste stimulus by the experimental animals, they fail to sufficiently address the taste-aversion issue. Had the deficient animals formed an association with their diet and some particular flavor and then subsequently displayed an aversion to the associated flavor while exhibiting a preference for the novel taste, stronger and more direct support could would have been given to the theory at hand.

In order that the learned taste-aversion theory be upheld by the present experiment,

subjects which had saccharin available as a fluid during the dietary exposure phase should display an aversion to the saccharin solution and a preference for water during subsequent two-bottle preference tests. The converse pattern of preference test results should be displayed by animals administered water during the dietary exposure phase.

Method

Subjects. Three litters of Holtzman-derived rats born and raised in the Emporia State University vivarium served as subjects. At birth the three litters were randomly culled to 12 animals each.

Apparatus. All testing was conducted in the home cage. The same sipper tubes and sippers that were used in Experiment 1 were employed in the present experiment.

Procedure. Upon weaning at 28 days of age, each rat was randomly assigned to 1 of 2 equal-sized groups (n = 18), and placed in an individual cage. The distribution of male and female pups was equated as much as possible between the two groups.

Due to the substantial amount of waste involved in maintaining the experimental animals on the soupy grits mixture in the first

experiment, Experiment 2 involved the use of dried grits cakes. These cakes were prepared by mixing 50 ml of tap water per 226.8 g of Quaker Instant Grits. This mixture was placed on a cookie sheet and baked at 325° for one and one-half hours, removed and cut into .60 cm squares. The squares were placed back on the cookie sheet and baked for an additional one and one-half hours or until they were thoroughly dry.

Subjects from both groups in Experiment 2 were maintained for six weeks from weaning on the grits diet. The two groups differed only in terms of their available fluids. Group WAT had plain tap water freely available during this period, while Group SAC, had free access to a .15% w/v sodium saccharin and water solution.

Twenty-four hours following the completion of the diet exposure phase, each animal was placed on 24 hours fluid deprivation. At the close of the fluid deprivation period, the animals were given the first, 15-minute, two-bottle preference test. Preference Test 2 was conducted 24 hours later.

Results

The preference-test data were converted to group mean consumption ratios as described in

Experiment 1, and are presented in Figure 2.

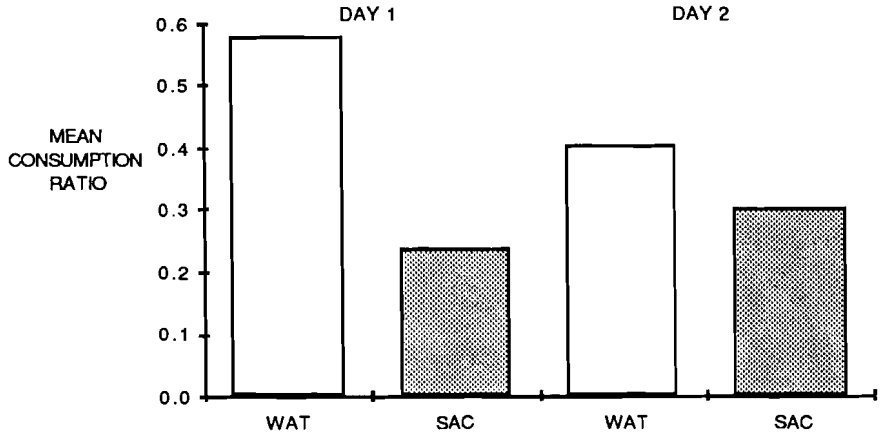

Figure 2. Group mean consumption ratios, calculated by dividing the amount of the target fluid (saccharin) consumed by the total amount of fluid consumed, for each of the 2 days of preference testing.

Analysis of variance performed on these data yielded significant groups, $F(1,30) = 30.96$, $p < .001$, and groups x days, $F(2,60) = 14.69$, $p < .001$, effects. Newman-Keuls tests performed on these data revealed that Group SAC animals had significantly ($p < .01$) lower consumption ratios than did those from Group WAT on both days of preference testing. Further, the scores of Group WAT animals were significantly ($p < .01$)

higher on the first of the two days of testing than they were on the second.

Discussion

The results from this experiment are able to more directly support the Rozin and Rodgers (1967) taste-aversion hypothesis. For subjects from each group, the preferred fluid during preference testing was the novel one (i.e., the animals displayed neophilia). Additionally, the depressed saccharin intake exhibited by Group SAC subjects reflects the strength of the aversion formed by those animals to that familiar taste. This observation, together with the higher consumption ratios displayed by group WAT animals, exemplifies the neophilic effect.

While Experiment 2 was able to successfully address the both issues--a neophilic preference for a new/unfamiliar taste, and an established aversion to familiar stimuli--a stronger case for the Rozin and Rodgers (1967) theory with regard to protein- and tryptophan-deprived animals remains to be made. Such support would be better and more clearly established if two flavor pairings were made during the dietary exposure phase, one with the illness-producing diet, and the other with a more balanced safe diet. The presence of both fluids during a

preference testing phase, then, would allow the animals to avoid the illness-associated fluid and opt for the safer flavor.

CHAPTER FOUR

Experiment 3

The results from Experiment 2 lend additional support to the taste-aversion account of neophilia. In order to more directly extend this theory to the grits-raised animals employed in the first two experiments, the third experiment was designed such that two distinct flavor parings could be formed with different foods from two experimental phases. The first fluid would be available during a dietary exposure phase in which normal laboratory chow was present. This "safe" period would allow for associations to be made between the nutritionally balanced laboratory chow and the first flavor. Subsequent to this safe period, the rats would then be subjected to a dietary deficiency (grits) phase during which a second flavor would be employed. Rozin and Rodgers (1967) would predict that the illness associated with the second phase would result in the formation of a taste-aversion to its accompanying fluid flavor. Due to the establishment of this aversion, the animals, when offered the choice, would consume more of the safe fluid. The third experiment was designed to test these predictions.

Method

Subjects. Two litters of Holtzman-derived rats, born and raised in the Emporia State University vivarium served as subjects.

Apparatus. As with the first two experiments, all testing took place in the home cage, and involved the use of the same sipper tubes and sippers.

Procedure. The rat pups remained with their respective dams from birth until weaning at age 21 days. Upon weaning, each animal was randomly assigned to one of two equal-sized (n = 8) groups. The distribution of males and females to the two groups was balanced as far as possible.

All subjects were fed freely-available Purina Laboratory Chow for the 45 days immediately following weaning/group assignment. Rats in group SAC-SAL received free access to saccharin solution (.15% w/v) during this time, while animals in group SAL-SAC were given free access to a saline solution (.09% w/v)--thus, the fluid presentation sequence for the two groups was counterbalanced.

For the 30 days subsequent to this first, safe, phase, both groups were placed on the protein- and tryptophan-deficient grits-cakes

diet, and the fluids for each group were switched. Hence, any illness associated with the grits diet should be paired with the specific fluid available during dietary exposure. Thus, group SAC-SAL animals would be predicted to form taste-aversions to the grits-associated saline solution, while the SAL-SAC animals would be expected to form aversions to the saccharin solution.

The 12 days following the dietary exposure phase of this experiment were used as a maintenance period. During this time all animals were maintained on freely available laboratory chow and plain tap water, thus allowing them to recover from the illness produced by exposure to the grits diet.

For 24 hours following the maintenance period, all animals were water deprived. At the conclusion of this deprivation period, the first of three, daily two-bottle preference tests was begun. The same procedure for testing that was used in Experiments 1 and 2 was used for the present experiment, except that saline was used in place of the water. Hence, the saccharin and saline flavors which accompanied the first two phases of Experiment 3 were pitted against one

another. As with the first two experiments, consumption was measured to the nearest .50 ml.

Results

Mean consumption ratios were calculated for both groups for the three days of preference testing by dividing the amount of saccharin consumed by the total amount of fluid (saccharin + saline) consumed. Ratios below .50 reflect a saccharin aversion, while those greater than .50 denote a saline aversion. The results of Experiment 3 preference tests are shown in Figure 3.

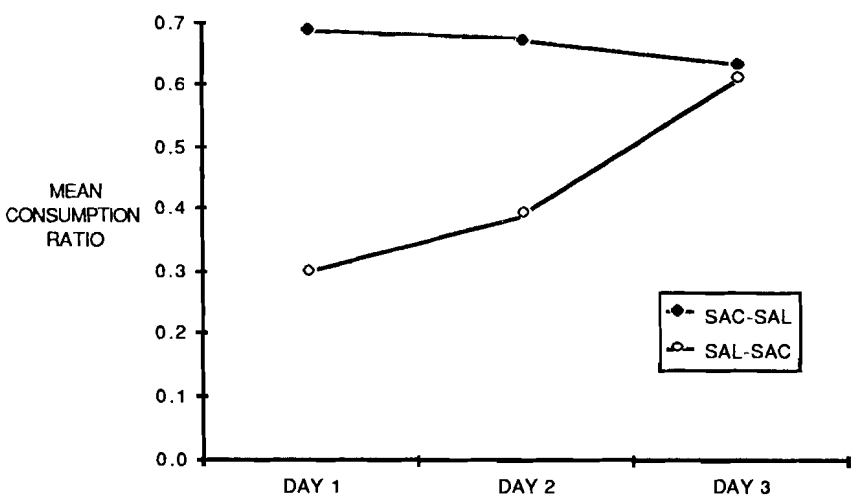

Figure 3. Group mean consumption ratios, calculated by dividing the amount of the target fluid (saccharin) consumed by the total amount of fluid consumed, for each of the 3 days of testing.

Analysis of the consumption ratio data yielded significance for the groups, $F(1,14) = 9.66$, $p < .01$, and days, $F(2,28) = 4.23$, $p < .05$, effects. As the groups respective ratios were appropriately above or below .50, the present experiment also resulted in neophilia. Newman-Keuls tests revealed that the consumption ratios for Group SAC-SAL were significantly ($p < .01$) higher than those for Group SAL-SAC for the first and second days of preference testing. The two groups did not differ dependably by the third day of testing. Further, it was found that the ratios for Group SAL-SAC from Day 3 were significantly higher ($p < .05$) than those from Day 1.

Discussion

Animals from both groups displayed marked aversions to the fluid that was available throughout the dietary-restriction phase. Given that the means from the two counterbalanced groups are appropriately above or below the .50 mark, the data from this experiment reflect preferences for the safe fluid resultant from aversions to the illness-associated fluid.

CHAPTER FIVE

General Discussion

Three experiments were conducted to test the learned taste-aversion account of neophilia proposed by Rozin and Rodgers (1967). Rats in the first experiment that were exposed to the protein- and tryptophan-deficient grits diet subsequently displayed a significantly greater preference for a novel peppermint flavor than did animals maintained on laboratory chow. However, the differences between the two groups only lasted through the first day of testing.

More lasting neophilic effects (i.e., 2 days versus 1 day) were shown by subjects from the second experiment when a specific taste was paired with the dietary deficiency and that taste was pitted against an unfamiliar flavor (saccharin for Group WAT animals, water for Group SAC animals). As these results are in line with the Rozin and Rodgers (1967) hypothesis, the learned taste-aversion explanation seems well qualified to handle these data.

Experiment 3 was designed such that all animals would be exposed to both fluids prior to preference testing. The fully-crossed experimental design used two counterbalanced

groups. One group's "safe" fluid was the other group's illness-associated fluid, and vice versa. By employing both fluids during preference testing, animals from each group were able to display aversions to their respective illness-associated fluids.

Though there are other explanations for the data from these three experiments, the learned taste-aversion model is best qualified for dealing with the full extent of the data. More specifically, Richter (1936) would claim that the animals were consuming the new flavors due to a craving for the missing vitamin or protein. While this claim might explain the initial sampling, it does not sufficiently discuss the rats resistance to the familiar, illness associated flavor. Had the animals merely been searching for a missing ingredient, they should have returned to the familiar diet upon satisfaction of the craving unless the malaise brought on by the grits diet was still in effect. By continuing the maintenance phase in Experiment 3 for 12 days following exposure to the grits-diet phase, it is reasonable to assume that recovery from the deficiency had indeed occurred. Given this return to a balanced nutritional state, the need to crave a missing

ingredient should have passed, and with it the animals tendency to display neophilic preferences.

Clearly this did not occur. One interpretation of these findings might be that the animals learned a preference during the safe period, and nothing during the dietary deficiency period. In light of the results from Experiments 1 and 2, though, a more parsimonious explanation is that the animals established aversions to their respective illness-paired flavors, and that these pairings resulted in a preference for the previously encountered safe fluid. This explanation is in line with the Rozin and Rodgers (1967) hypothesis.

In sum, Experiments 1 and 2 showed that the animals had formed aversions to familiar, illness-asociated fluids, while Experiment 3 marked the animals' ability to differentiate between the illness-paired flavor and a previously encountered safe fluid. Thus, the present series of experiments has consistently produced neophilic preferences in animals administered a protein- and tryptophan-deficient grits diet.

One substantial problem with regard to neophilia, however, remains to be resolved.

Specifically, the animals in these experiments have appropriately chosen the fluid that was not present during a dietary deficiency phase. The experimental designs employed, however, do not sufficiently explain the nature of that choice. Future research in this area will need to focus on whether this selection stems from (a) a reaction brought on by an established aversion to the illness-associated fluid, or (b) a conditioned preference for the alternate or previously encountered safe fluid.

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