A COMPARATIVE STUDY OF THE TASTE REACTIONS OF JAPANESE QUAIL, MONGOLIAN GERBILS, AND SQUIRREL MONKEYS TO SIX METALLIC CHLORIDES

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PREFACE

This investigation is concerned with taste-preference behavior. Taste-preference behavior is a term which signifies the differential act of oral ingestion of one substance instead of another by an organism. This behavior is controlled by a number of factors; one of which is stimulation of the oral taste receptors. The major purpose of this study is to clarify whether or not there is a possible similarity in taste-preference behavior among different species.

The author wishes to express his appreciation to a number of people who have helped make this study possible. A very large debt is owed to Dr. Arthur E. Harriman, who served as the thesis adviser. Dr. Harriman gave generously of his time and knowledge in making this investigation possible. I would also like to acknowledge the aid of Dr. Larry T. Brown, who made available the squirrel monkeys used in this study, and who served on the thesis committee. Appreciation is extended to Dr. David M. Shoemaker, who helped extensively in the analysis of the results of this investigation. Finally, I would like to thank my wife, Louise, for her aid in testing subjects and in typing the manuscript. Without her encouragement this study would not have been possible.

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CHAPTER I

INTRODUCTION

Historical Review

Studies on the sense of taste have a long history (Kare and Ficken, 1963), but truly comparative studies have been undertaken only in recent years. Parker, the pioneer of comparative studies on taste (Kare and Ficken, 1963), examined the chemical senses in a variety of invertebrates and fish. He found that simple organisms, such as the amoeba, show a general irritability to chemical stimulation, whereas at higher phylogenetic levels, this irritability differentiates into a common chemical sensitivity and a tactile sensitivity. At still higher levels, the common chemical sensitivity is further differentiated, and a localization and specialization of receptors is found in the oral cavity. These chemoreceptors are sensitive to extremely diluted chemical compounds and have a great specificity for chemical structure. The localization of chemoreceptors in the mouth and pharynx is also found to be a characteristic of the "air inhabiting" vertebrates (Kare, 1961).

A comparative approach has been used in some of the electrophysiological studies on the sense of taste (Appelberg, 1958; Beidler, Fishman, and Hardiman, 1955; Pfaffmann, 1953, 1955; and Zotterman, 1956). These studies have been mainly concerned with comparing discrimination thresholds and neural response patterns to different substances in various species. The comparative approach has been used in a few behavioral studies of taste. These studies have tried to determine the preference-aversion drinking functions for different substances by various species. It has proven difficult to correlate these two areas of research (Kare and Ficken, 1963; Carpenter, 1956).

One reason it has been hard to correlate electrophysiological and behavioral research findings is that there are few comparative studies of taste-preference behavior. This is due to a number of reasons. First, the difficulties inherent in the maintenance and testing of several species are enormous. Considerable amounts of laboratory space and equipment are needed. The second reason has been the concern of earlier investigators for the types of stimuli used and not the reactions of various organisms to the stimuli. In fact, most organisms have been assumed to respond like man to various taste stimuli (Kare and Ficken, 1963). A third reason has been the lack of investigations concerned with evolutionary problems in taste behavior. The use of evolutionary principles in behavioral research is still rather new. As a result, the study of species differences and similarities in taste-preference behavior is largely an unexplored area.

A Selected Review of the Literature

According to Carpenter (1956), his was the first comparative investigation of taste-preference behavior. He studied two groups of five rabbits, one group of nine hamsters, and one group of eight cats. The same taste stimuli and two-bottle preference tests were used for all species. He presented each test solution opposite tap water for 48 hours and reversed the position of the bottles on the cages every 24 hours. At the end of a 48-hour period, the test solutions were increased in concentration by a 1/3 log molar step. The following compounds were used: NaCl, KCl, sucrose, sodium saccharin, and quinine HCl. The initial concentrations were chosen so that they were below taste threshold for the animals. The ascending concentrations in each test series were ended when the animals avoided the test solution by drinking less of the test solution than at the previous concentration.

Carpenter claimed that significant species differences were noted in response to NaCl, sucrose, and sodium saccharin, whereas KCl and quinine HCl produced similar responses in the three species. Carpenter classified a particular compound as "preferred" if the test solution of that compound was consumed in significantly greater quantities than tap water at any concentration. A compound was classified as "avoided" if the intake of the test solution of that compound decreased with the test solution's increase in concentration. A compound was classified as "not discriminated" if the test solution intake and water intake were equal. On the basis of these classifications of compounds, Carpenter found that cats preferred NaCl but avoided quinine HCl, sodium saccharin, and KCl, and did not discriminate sucrose from water. Hamsters preferred sucrose and sodium saccharin, but avoided quinine HCl, NaCl, and KCl. Rabbits preferred sucrose, sodium saccharin, and NaCl, but avoided quinine HCl and KCl.

Duncan (1962) performed a study comparing the taste preferences of feral pigeons (Columba livia var. Gmelin) and white rats by the use of the method of single stimuli. The experiment was planned on a latin squares basis, so that in each daily test all concentrations of a particular compound were used. Tap water was included as zero concentration, and the volume drunk served as a reference point for the preference or rejection of the test solutions.

Duncan reported that the NaCl taste-preference data for the feral pigeons agreed with the data for rats previously obtained from the use of a similar technique. The consumption of 0.008M and 0.017M NaCl was not significantly different from the consumption of tap water, but a significant increase in comsumption over water was found at 0.043M and 0.085M NaCl. Solutions of 0.14M NaCl or stronger were consumed in less quantities than water. The pigeons and rats were also tested with various concentrations of NH_4Cl, KCl, and $CaCl_2$. The consumption by the pigeons of NH_4Cl was similar to that of NaCl, but the rejection of NH_4Cl began at higher molar concentrations than for NaCl. The pigeons showed an overall aversion to solutions of KCl, and $CaCl_2$, but a small preference was shown for 0.02M KCl and 0.30M $CaCl_2$. The stimulative efficiencies of the cations were determined by the concentration of the cation compound at which initial maximum rejection was shown. Those cation compounds which produced maximum rejection at low concentrations were presumed to have the more stimulative efficient cation. The order of the stimulative efficiencies of the cations as chlorides for the pigeons was in the following sequence: $Na_4^+ > NH_4^+ > K^+ > Ca^{++}$. The order for the rats was in the following sequence: $NH_4^+ > Ca^{++} > K^+$. Duncan felt that neither species produced an order of stimulative efficiencies which agreed with the order of cation mobility. The order of cation mobility, defined as ionic conductance in mhos at 25°C, is $NH_4^+ > K^+ > Ca^{++} > Na^+$. Duncan stated that the only similarity in taste preference behavior between rats and pigeons is their similar preference for isotonic NaCl.

Kare (1961; Kare and Ficken, 1963) has presented a series of arguments for the notion that each species lives in an "isolated taste world." Kare states that there are differences of degree among individuals of a particular species, and that there are absolute differences among species. To support this contention, Kare and Ficken have presented data on the consumption of various types of sugars in two-bottle preference tests with distilled water as the comparison solution for chicks, calves, and rats. They reported that chicks rejected xylose at all concentrations, that calves rejected maltose at all concentrations, and that rats rejected xylose at high concentrations. Kare and Ficken have also reported that calves were indifferent to various concentrations of saccharin, and that rats preferred saccharin at low concentrations, but avoided saccharin at high concentrations. Kare and Ficken conclude that the differential preference of various species for different sweet-tasting substance (to man) points to an absolute difference among species in terms of the various species' taste-preference behavior. They have further reported that different individuals in a particular species exhibit differential preferences for the different sweet-tasting substances, but that the interindividual preferences are not so great in magnitude as the differential preferences among species. Kare and Ficken conclude that the smaller magnitude of the differential preferences of individuals supports the idea of differences of degree in taste-preference behavior among individuals of the same species.

Fisher, Pfaffmann, and Brown (1965) have reported a study of dulcin and sodium saccharin taste preference in squirrel monkeys (Saimiri Sciureus), albino rats, and humans. They used the two-bottle preference method with distilled water as the comparison solution in testing the animals. They found that squirrel monkeys had greater preference for dulcin than sodium saccharin, and that rats rejected dulcin but preferred sodium saccharin. The human subjects "liked" the sweet taste of dulcin and sodium saccharin.

The studies with sweet-tasting substances (to humans) seem to indicate that different species do have unique "taste worlds," but a question can be raised about this conclusion. For one thing, most of the studies cited were performed on only one or a small number of species at a time, so that several different studies have been combined to make comparative statements. The possibility of experimental conditions varying among species was great. Another problem has been the lack of the use of statistical analyses on the results. Most of the conclusions have been based upon graphical representation of the results. Whether or not the results were significant in relation to the error variability of a particular study is questionable, especially since Kare and Ficken (1963) have reported that large differences among individuals are not unusual in taste-preference studies.

An area of investigation which is related to the study of taste preference is that of taste thresholds. A major objective of these latter studies has been to discover the acceptance and rejection thresholds as well as the absolute thresholds in different species for various compounds.

Richter and MacLean (1939), in a study of the taste thresholds for NaCl, found the threshold for recognizing the salty taste to be about 0.065 percent (percent weight of NaCl to volume of distilled water) for humans. This value was in close agreement with the preference threshold in rats (0.055 percent). On the basis of this study, Richter claimed that humans and rats have nearly the same salt taste thresholds.

It should be pointed out that rats and humans respond differentially to NaCl test solutions and distilled water at much lower concentrations. Richter and MacLean reported that the human subjects described the NaCl solutions as tasting "bitter" and "sweet" before the subjects recognized a "salty" taste. Later studies (Harriman and MacLeod, 1953; Carr, 1956), using a forced-choice procedure, have reported that rats can discriminate NaCl solutions from distilled water at very low concentrations. Carr obtained threshold values of 0.002 to 0.019 percent, and those reported by Harriman and MacLeod were even lower (0.000025 to 0.002 percent).

Schwartzbaum and Wilson (1961) investigated the absolute threshold for NaCl in rhesus monkeys. They used a non-preference method and found that the average minimal concentration of NaCl that could be discriminated from distilled water was 0.028 percent. They stated that this value was close to the average concentration of NaCl (0.016 percent) found by Richter and MacLean (1939) for the initial recognition by man of a NaCl solution from distilled water. From a comparative point of view, Schwartzbaum and Wilson conclude that the taste threshold of the rhesus monkey for NaCl is in the same range as that of man and above that of the rat.

A number of studies have been performed which have investigated the acceptance and rejection thresholds for various compounds in a number of species. Frings (1945) determined the rejection thresholds in caterpillars of the cecropia moth, <u>Samia</u> <u>cecropia</u>, for a number of compounds, presented as drops of solutions on leaves of the food plant (sycamore). He defined the rejection threshold as the concentration of the compound midway between the highest concentration accepted and the lowest concentration rejected. Frings found that the order of stimulative efficiencies of the cations as chlorides, determined by the reciprocals of the normal concentrations of the chlorides at the rejection thresholds, gave the following series for the caterpillar of the cecropia moth: $NH_4^+ = K^+ > Ca^{++} > Na^+ > Li^+$. He concludes that this series is the order of ionic mobilities (limiting equivalent ionic conductances which are directly proportional to the ionic mobilities) to which the stimulative efficiencies seem to be related.

In a later study, Frings (1946) examined the acceptance and rejection thresholds in the cockroach, *Periplaneta americana*, for a number of electrolytes. The reciprocals of the rejection threshold normalities were used to produce the following order of stimulative efficiencies of the cations in uniform anion series for the cockroach: $NH_4^+ > Rb^+ = Cs^+ \geq K^+ > Sr^{++} = Ca^{++} > Mg^{++} > Na^+ > Li^+$. The order of the stimulative efficiencies of the cations was again the same as the ionic mobilities of the cations and paralleled the findings with caterpillars.

Frings and O'Neal (1946) have studied the rejection thresholds in the female horsefly, *Tabanus sulcifrons*, for various electrolytes. Median rejection thresholds were obtained by determining the concentration of the electrolyte in 0.2M sucrose at which 50 percent of the animals rejected the solutions. The rejection thresholds were nearly the same as in previous insect studies. The order of stimulative efficiencies of the cations as chlorides was as follows for the horsefly: $NH_4^+ > K^+ > Na^+ > Li^+$.

Frings (1948) examined the rejection thresholds in caterpillars of *Eacles imperialis* and laboratory-bred rabbits for five metallic chlorides, and he also examined the absolute sensory thresholds in man for the same metallic chlorides. The order of stimulative efficiencies of the cations, as measured by the reciprocals of the mean rejection threshold normalities, for the caterpillars was as follows: $NH_4^+ \geq K^+ > Ca^{++} > Na^+ > Li^+$. For the rabbits, the order was as follows: $NH_4^+ \geq K^+ > Ca^{++} = Na^+ > Li^+$. For man, the order was as follows: $NH_4^+ \geq K^+ > Ca^{++} > Na^+$. The order of stimulative efficiencies of the cations for man was determined from the absolute sensory threshold normalities of the chlorides.

Hamrum (1953) studied the median rejection thresholds in the bob-white quail (Colinus virginianus virginianus) for five metallic chlorides. The median rejection threshold, as defined by Hamrum, was that concentration at which the intake of the test solution was 25 percent of the total intake (two-bottle preference test). Hamrum reported that the quail rejected the cations as chlorides in the following order: $Li^+ \geq NH_4^+ > K^+ > Ca^{++} > Na^+$.

Frings (1945, 1946, 1948; Fring and O'Neal, 1946) has presented evidence that the order of stimulative efficiencies of cations, as determined by rejection and absolute sensory thresholds, is similar in different species. The similarity of orders of stimulative efficiencies of cations in different species is assumed by Frings (1946) to be due to the ionic mobilities of the cations. The mechanism of taste sensation is also assumed by Frings (1946, 1951) to be based on the ionic mobilities (penetration power) of different sapid substances (taste-spectrum hypothesis). Due to the similarity of the orders of stimulative efficiencies of cations across species, Frings (1946, 1951) has assumed that there could be a similarity among species in terms of taste sensations based on the operation of the ionic mobility taste-mechanism.

From Frings' theorizing, it is difficult to say that there would be a continuity among species in terms of taste-preference behavior. Whether or not a similarity of taste sensations among species would lead to a similarity of taste-preference behavior among species is questionable due to the possible operation of postingestive factors in taste-preference behavior. In addition, there is evidence that the order of stimulative efficiencies of cations, upon which Frings bases his arguments, is not the same across species (Duncan, 1962; Hamrum, 1953).

It should be apparent that the comparative and related literature does not result in any concise formulation. Some investigators (Carpenter, 1956; Duncan, 1962; Frings, 1945, 1946, 1948; Frings and O'Neal, 1946; Hamrum, 1953, Richter and MacLean, 1939) have found some continuity across species in terms of their taste-preference behavior to various stimuli. Others (Carpenter, 1956; Kare, 1961; Kare and Ficken, 1963; Fisher, Pfaffmann, and Brown, 1965; Schwartzbaum and Wilson, 1961) have not. Those who did find some continuity often used various concentrations of metallic chlorides as their stimuli. Those who failed to find continuity often used various concentrations of sugars or sweet-tasting (to man) substances as test-solution stimuli. One general finding has been that most metallic chlorides, such as NaCl, KCl, NH_4Cl, etc., are not preferred by most organisms and that with increasing concentration the intake of these chloride solutions decreases relative to the total fluid intake per unit of time. The particular preference function with regard to concentrations usually depends on the type of chloride, but all organisms tested have had a rejection threshold for metallic chlorides at some concentration. NaCl seems unique, because at low concentrations (below 0.15M) it is preferred by some species (Carpenter, 1956; Duncan, 1962). KCl, NH_4Cl, and $CaCl_2$ evoke slight preferences at low concentrations, but this effect has not been large when found (Duncan, 1962). The orders of stimulative efficiencies of cations as reported by Frings (1945, 1946, 1948; Frings and O'Neal, 1946) have been identical among the species he has examined, but the orders of stimulative efficiencies of cations reported by Duncan (1962) and Hamrum (1953) do not agree with Frings' findings. There has been no consistent finding of preference or aversion to the various sugar compounds or sweet-tasting substances across various species (Kare, 1961; Kare and Ficken, 1963).

It should be noted that one of the factors that makes it difficult to make many comparative statements is that different species have been studied using different methods. The use of different methods confounds differences among species with differences in experimental technique. This confounding was Carpenter's (1956) reason for declaring his study to be the first comparative investigation of taste-preference behavior. Since then, no study has fully met the requirements of comparative investigation. Therefore, it is extremely dangerous to make statements and generalizations about unique species effects until further work has been done. One of the major reasons for conducting this study was to provide more information upon which generalizations about taste-preference behavior across species may be made.

The Problem to be Investigated

There have been two roughly opposed notions appearing in the literature. One notion states that each species lives in an "absolute species specific taste world" (Kare, 1961; Kare and Ficken, 1963). The other notion declares that there might be an underlying species-to-species continuity in taste sensation. Frings (1946, 1948, 1951) embodies this last notion in his "taste-spectrum" hypothesis. In this hypothesis, Frings tries to relate a physical characteristic (ionic mobilities) of certain substances, via their stimulative efficiencies, to their taste sensations or rejection thresholds across different species.

Due to the fact that neither notion is adequately stated in terms of taste-preference behavior, it is impossible to formulate an adequate hypothesis to be tested from either notion. Therefore, the basic problem for investigation becomes one of testing different species to see what effects the species variable has on taste-preference behavior.

Specifically, this investigation concerns itself with the comparative study of the taste-preference behavior of the squirrel monkey (Samiri sciureus), the Japanese quail (Coturnix coturnix japonica), and the Mongolian gerbil (Gerbillus gerbillus) to NaCl, KCl, NH_4Cl, $MgCl_2$, $CaCl_2$, and $SrCl_2$ at four molar concentrations (0.01, 0.05, 0.10, and 0.15M) per substance. None of these species has been used in reported investigations of taste-preference behavior to metallic chlorides; therefore, a secondary concern of this investigation was to discover the reactions of the individual species to these chlorides.

The following were the specific problems to be investigated. First, what is the effect of the species to be tested on taste-preference behavior for the metallic chlorides? Second, what are the effects of the chlorides and their concentrations on such behavior in these species? Lastly, is there any unique effect on taste-preference behavior due to the combination of these species, chlorides, and concentrations? Answers to these questions should help provide information upon which theories of taste-preference behavior can be developed.

CHAPTER II

METHODOLOGY

Selection of Species and Stimuli

It was desired to use three species of animals that are fairly widely separated on the phylogenetic scale. The Psychology Department at Oklahoma State University had a colony of each of the following species: squirrel monkeys (*Saimiri sciureus*), Japanese quail (*Coturnix coturnix japonica*), and Mongolian gerbils (*Gerbillus gerbillus*). These three species seemed to meet the above criteria.

It was also desired to use species which could be adequately studied with similar preference-testing techniques. Previous work in the Psychology Department laboratories of Oklahoma State University had shown this to be possible with the selected species. All members of each species could be caged individually and were found to respond satisfactorily in the two-bottle preference test situation. Kelleher, Gill, Riddle, and Cook (1963) note that the squirrel monkey is a useful experimental subject for both behavioral and pharmacological studies. Reese and Reese (1962) are of the opinion that *C. coturnix* would be a useful experimental animal for comparative studies. The previous work that has been done with gerbils in the Psychology Department laboratories of Oklahoma State University has proven them to be good experimental subjects for taste-preference studies.

The decision to use various metallic chlorides as stimulus solutions was based on the fact that a majority of comparative and comparative-related studies have been performed with these chlorides. Another factor was that none of the species have ever been studied with regard to their taste-preference behavior for these chlorides.

The criteria for the selection of the particular metallic chlorides to be used were as follows. First, they should be colorless in solution with water. This was based upon the fact that Straka (1966) has found a drinking preference in pigeons for blue-colored water over red or green water, and that Brindley (1965) has found a color preference in Japanese quail in the two-bottle preference test situation. Second, the chlorides should be nonpoisonous at hypotonic or isotonic concentrations. The following metallic chlorides met these criteria: NaCl, $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, KCl, NH_4Cl, and $SrCl_2 \cdot 6H_2O$.

It has been stated (Carpenter, 1956) that for hypertonic solutions taste-preference behavior is probably determined by both postingestive and oral stimulation (taste) factors, whereas for isotonic and hypotonic solutions taste-preference behavior is probably determined mainly by oral stimulation (taste) factors. The present investigation was limited to taste-preference behavior which was assumed to be determined by oral factors. Therefore, the concentrations selected for use are either hypotonic or isotonic. The molar concentrations of each of the selected chlorides were the following: 0.01, 0.05, 0.10, and 0.15M.

Description of Subjects and Apparatus

There were 12 subjects chosen from the colony of each of the selected species. The gerbils were all female. There were nine female and three male quail, and six male and six female squirrel monkeys. The quail were 138 days old at the start of the experiment, and the gerbils were 268 days old. The squirrel monkeys were of unknown age because all were feral animals. It is difficult to estimate the age of individual squirrel monkeys because their life span has not been established (Kelleher, _et al._, 1963). In general, the male squirrel monkeys were younger looking and had been acquired more recently than the females.

The quail were hatched from eggs provided by the Oklahoma State University Poultry Department and had been in one previous taste-preference study with NaCl. The gerbils were acquired as pups, and had been in previous taste-preference studies, but none involving metallic chlorides. Both species were laboratory reared. The squirrel monkeys had been captured in the wild as adults. They had been used in previous studies, but were naive with regard to taste-preference work.

All subjects were in good health, except one female squirrel monkey who had lost her teeth. Otherwise, she was in fairly good condition. At the start of the study, the squirrel monkeys ranged in weight from 405 gms. to 1000 gms. with a mean weight of 711 gms., _S.D._=208.9 gms.; the quail ranged in weight from 100 gms. to 134 gms. with a mean weight of 115 gms., _S.D._=8.9 gms.; the gerbils ranged in weight from 60 gms. to 87 gms. with a mean weight of 72 gms., _S.D._=8.6 gms. All the female quail were in autumnal molt during the entire investigation. As a result, their total fluid intake was quite similar to that of the males.

All subjects were maintained in individual cages. For the quail, the cages measured 24 inches long by 16 inches wide by 13.5 inches deep. The gerbil cages were 15 inches long by 9 inches wide by 7.5 inches deep. The squirrel monkeys were housed in double cages constructed by placing 1/4-inch hardware cloth down the middle of a large cage. Each squirrel monkey lived in a cage that measured 20 inches long by 10 inches wide by 30 inches deep. Each individual cage was provided with a perch for the squirrel monkey to sit upon, and these perches were located 14 inches above the bottom of cage and 10 inches from the front of the cage.

Two rooms were used to house the animals. The gerbils and quail were in one room while the squirrel monkeys were housed in a larger room next door. Both rooms were on the same air-conditioning system which provided fresh outside air at all times. Both rooms had the fluorescent lighting controlled automatically, with the lights on from 8:00 A.M. to 8:00 P.M. each day. The temperature was maintained at 25.1°C, S.D.= 1.63°C, and the level of relative humidity was 32.7 percent, S.D.=8.74 percent, throughout the study.

All subjects were maintained on an ad lib food schedule. The quail were fed on Purina Game Bird Startena (Medicated). The gerbils were fed Purina Laboratory Rat Chow, and the squirrel monkeys were fed Rockland Laboratory Primate Diet. All feed was in a dry form. The squirrel monkey diet was supplemented with two fresh orange sections every other day. Two of the female squirrel monkeys needed their food cubes soaked. It was found that a maximum of 80 gms. water was contained in the 200 gms. of soaked food given to these two subjects. The quail had their feed presented in containers that measured seven and 1/4 inches in diameter with nine one-inch circular holes in the container cover. The food containers were placed at the rear areas of the cages. The gerbils received their food on the floor of their cages, as did the squirrel monkeys. Fresh food cubes were given to the squirrel monkeys everyday.

All fluids were presented to the subjects in two fluid containers. Chick waterers with inverted pint jars were used with the quail. The waterers measured five and 1/2 inches in diameter with a one-inch circular water trough. These were placed about one and 1/2 inches from the front of the cage and about six inches from the sides about the midline of the cage. As a result of this placement, the lips of the waterers nearly touched and provided a distance of approximately two inches between the water troughs. For the gerbils, fluids were presented in 125 ml. calibrated Wahmann drinking tubes. These were located approximately two and 1/2 inches and four and 1/2 inches from the left edge on the front of each cage and about two inches from the floor of each cage. The tubes were approximately two inches apart and protruded two inches into the cages. Fluids were presented to the squirrel monkeys in 250 ml. calibrated Wahmann drinking tubes. These were located approximately two inches and four inches from the left or right edge on the front of each individual's cage with approximately two inches between the ends of the tubes. They protruded two inches into the cage at about four inches above the perches. All drinking tubes were mounted securely to the cages.

Two-Bottle Preference Testing Procedure

Due to the amount of work involved, the quail and gerbils were tested before the squirrel monkeys. Testing was commenced with the squirrel monkeys after the termination of testing with the quail and gerbils. A 10-day habituation period was given to the quail and gerbils during which both fluid containers were filled with distilled water. The water was double distilled and stored in glass jugs. All subjects were weighed before the habituation period, and at the end of preference testing. The waterers and drinking tubes were reversed in position every 24 hours. Clean waterers and drinking tubes, filled with fresh distilled water, were provided every 48 hours during the habituation period for the quail and gerbils, and every 72 hours for the squirrel monkeys.

During the habituation period for the squirrel monkeys, it was noted that one of the subjects was responding to the drinking tubes by placing his food cubes in the tubes. This necessitated his elimination from the study, and the inclusion of an old female. As a result, she received only seven days of habituation. the other squirrel monkeys actually received 12 days of habituation because the drinking tubes leaked for two of the 12 days. The objectives of the habituation period were to acquaint all species with the equipment and to stabilize their fluid consumption.

Prior to the beginning of the 10-day habituation period, 12 gerbils and 12 quail were randomly chosen from larger populations and were randomly assigned to cages, which were numbered in consecutive order. The squirrel monkeys had already been assigned to living cages prior to the beginning of their habituation period, and no particular order of assignment had been followed except to pair a male and female together where possible. The individual squirrel monkey cages were numbered in consecutive order. Gerbils and quail were formed into pairs by combining Subjects #1 and #2 into one pair, Subjects #3 and #4 into another pair, and so forth through Subjects #11 and #12. The same procedure was followed with the squirrel monkeys, but due to the exclusion of three subjects, all members of each pair did not live next to each other. The pairing procedure resulted in six pairs of quail, six pairs of gerbils, and six pairs of squirrel monkeys. Prior to the end of the habituation periods, the six metallic chlorides were assigned to pairs through the use of a table of random numbers.

During preference testing, each subject was offered two fluid containers. One of them was filled with double-distilled water, and the other was filled with the appropriate concentration of the assigned metallic chloride. A six-day, two-bottle preference test then followed, during which readings (in milliliters for the gerbils and squirrel monkeys and grams for the quail) were taken every 24 hours. The fluid containers were reversed after each reading. The chloride solution containers were in the following order of position on the cages: left the first 24 hours, right the second 24 hours, left, right, left, right. The distilled water containers followed a reverse order of position: right, left, right, left, right, left. Reversal was used to balance the effects of position preferences. A separate six-day, two-bottle preference test was employed with each molar concentration used. As a result, there were four consecutive six-day, two-bottle preference tests. Each six-day test was at the next higher molar concentration than the preceding one. Preference testing took place over a 24-day period. Clean glassware and fresh solutions were provided every 72 hours.

The four molar concentrations (0.01, 0.05, 0.10, and 0.15M) of each metallic chloride were prepared volumetrically at 24°C from 1.00M stock solutions of NaCl, $SrCl_2$, $MgCl_2$, NH_4Cl, KCl, and $CaCl_2$. The stock solutions were prepared in two liter volumetric flasks from Baker's Analyzed Reageant Grade chemicals and double-distilled water. Each concentration of a chloride was prepared in two-liter lots with double-distilled water and stored in two-liter Erylenmeyer flasks. Two sets of lots were made. One set was for the gerbils and quail, and the other for the squirrel monkeys.

Summary of Procedure

The basic plan of investigation consisted of testing 12 subjects from each of three species (Mongolian gerbil, Japanese quail, and squirrel monkeys) for their taste-preference behavior to six metallic chlorides (NaCl, KCl, NH_4Cl, $MgCl_2$, $SrCl_2$, and $CaCl_2$) in a two-bottle preference test situation. Each chloride was assigned at random to a pair of subjects from each species. Each pair was tested for six days at each of four concentrations of the assigned chloride. The concentrations were administered in an ascending order (0.01M, 0.05M, 0.10M, and 0.15M). Readings were taken of the amount (milliliters or grams) of distilled water and chloride solution drunk at 24-hour intervals during each six-day test. The fluid containers were alternated in position after each reading. The raw data consisted of the percent preference for a chloride solution by each subject for a particular six-day test. This percent preference was computed for each subject by dividing the total amount of chloride solution consumed during the six-day test by the total amount of both chloride solution and distilled water consumed during the six-day test. This quotient was multiplied by 100 to give a percent value.

CHAPTER III

RESULTS

Statistical Design

The experimental procedure used in this investigation conforms to a three-factor, factorial design with repeated measures on one factor. All three factors were considered fixed. The first factor was factor A (species) which had three levels (Japanese quail, Mongolian gerbils, and squirrel monkeys). The second factor was factor B (metallic chlorides). There were six levels of this factor (NaCl, $SrCl_2$, NH_4Cl, KCl, $MgCl_2$, and $CaCl_2$). The third factor was factor C (molar concentrations) of which there were four levels (0.01, 0.05, 0.10, and 0.15M). Factor C was the one on which repeated measures were taken. Two subjects were assigned to each experimental unit. The main analysis was performed by means of an analysis-of-variance and is presented in Table I.

This design corresponds to Winer's (1962, pp. 337-348) three factor, factorial design with repeated measures on one factor (Case II). The basic assumptions required for an appropriate analysis are the usual analysis-of-variance assumptions with the additional assumption of homogeneity and equality of variance-covariance matrices for the sample data. The assumption of equality and homogeneity of variance-covariance matrices applies to the within subjects effects. These effects were tested by Box's test which assumes the variance-covariance assumptions not to be supported (Winer, 1962, p. 340).

Results

The main effects for chlorides ($p<0.05$) and concentrations ($p<0.001$), presented in Table I, were statistically significant. The interactions of species by concentrations ($p<0.01$) and chlorides by concentrations ($p<0.05$) were also significant. The main effects due to species were not significant, as were the species by chlorides interaction and the species by chlorides by concentrations interaction.

A test, reported in Table II, was made by using the Newman-Keuls procedure on the means of the main effects of both chlorides and concentrations. For the test on chlorides, only the NaCl mean was significantly ($p<0.05$) different from the other chloride means. For the test on concentrations, all the main effect means for concentrations were significantly ($p<0.01$) different from each other. The main effect means for the chlorides are plotted in Figure 1. It can be seen that NaCl had the highest mean percent preference value. This mean was significantly higher than was the case for the other five chloride means, but none of the other chloride means were significantly different from each other. Figure 2 is a plot of mean percent preference for concentrations. There is an almost perfect linear decrease in mean percent preference with increasing concentrations.

Due to the significant interaction effects found for the species by concentrations and the chlorides by concentrations interactions, an analysis of simple main effects was made on each interaction, and the results are reported in Table III and Table IV. For the species by concentrations interaction, only the concentration simple effects for the squirrel monkey condition were significant ($p<0.05$). The concentrations simple effects for the squirrel monkey condition can be seen in Figure 3

TABLE I

AOV: MAIN ANALYSIS

Source	df	SS	MS	F	P
Total	143	70,250.898			
Between Ss	35	36,301.264			
Species (A)	2	2,095.417	1,047.708	1.592	.75
Chlorides (B)	5	10,149.572	2,029.914	3.084	.05
AB	10	12,209.914	1,220.959	1.855	.75
Error (Between)	18	11,846.683	658.149		
Within Ss	108	33,949.634			
Concentrations (C)	3	16,880.458	5,626.819	70.619	.001 & .001*
AC	6	3,369.496	561.583	7.048	.001 & .01*
BC	15	4,565.918	304.395	3.820	.001 & .05*
ABC	30	4,831.144	161.038	2.021	.05 & .10*
Error (Within)	54	4,302.619	79.678		

* Significance value reached with Box's correction for heterogeneous and unequal variance-covariance matrices (Winer, 1962, p. 340).

TABLE II

TESTS ON CONCENTRATIONS AND CHLORIDE MAIN EFFECTS USING NEWMAN-KEULS PROCEDURE

Salts	$SrCl_2$	$MgCl_2$	NH_4Cl	$CaCl_2$	KCl	NaCl
Means	25.92	33.41	36.00	36.92	42.11	53.30
25.92		7.49	10.08	11.00	16.19	27.38*
33.41			2.59	3.51	8.70	19.89
36.00				0.92	6.11	17.30
36.92					5.19	16.38
42.11						11.19

$$S_{\bar{\bar{B}}} = \sqrt{\frac{\text{MS error between}}{npr}}$$

	r=	2	3	4	5	6
$S_{\bar{\bar{B}}} = 5.24$						
$q\ .95(r,\ 18)$		2.97	3.61	4.00	4.28	4.49
$S_{\bar{\bar{B}}} q\ .95(r,\ 18)$		15.56	18.92	20.96	22.43	23.53

* Significant difference at $p<0.05$

Concentrations	0.15	0.10	0.05	0.01
Means	24.10	33.64	40.15	53.88
24.10		9.54*	16.05*	29.78*
33.64			6.51*	20.24*
40.15				13.73*

$$S_{\bar{\bar{C}}} = \sqrt{\frac{\text{MS error within}}{npq}}$$

	r=	2	3	4
$S_{\bar{\bar{C}}} = 1.49$				
$q\ .99(r,\ 54)$		3.76	4.28	4.60
$S_{\bar{\bar{C}}}\ q\ .99(r,\ 54)$		5.60	6.38	6.85

* Significant difference at $p<0.01$

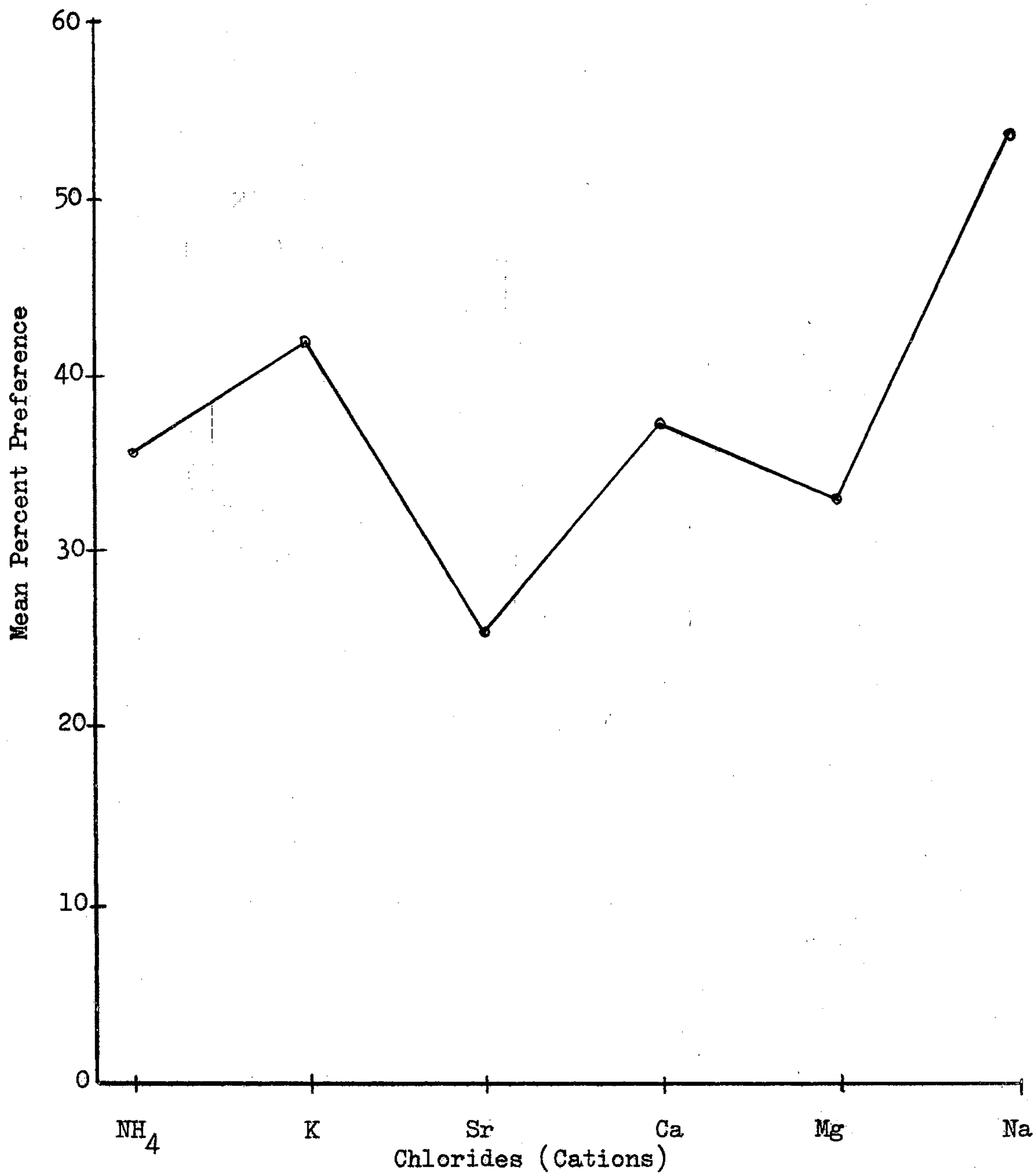

Figure 1. Mean Percent Preferences for Chlorides (Cations)

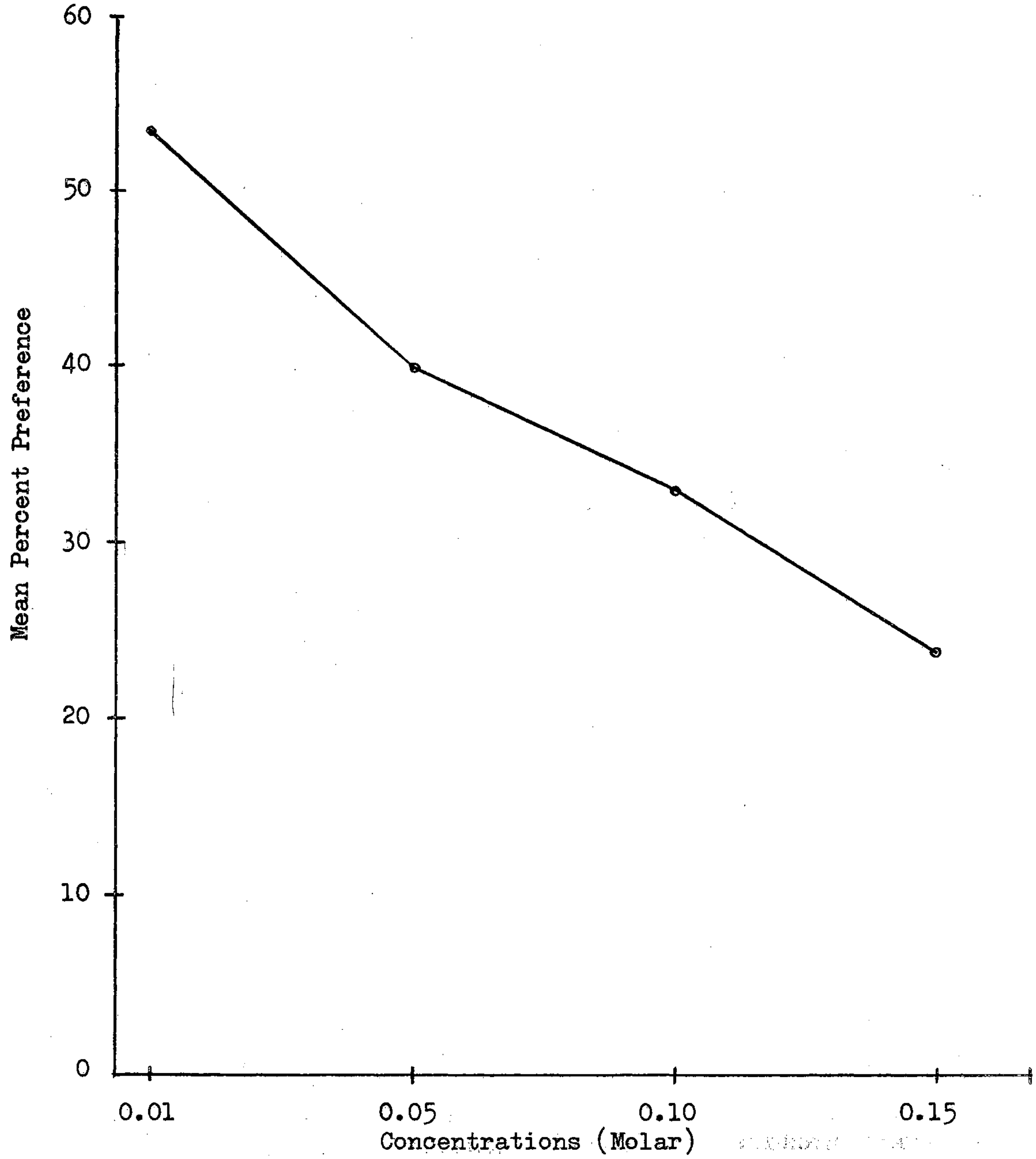

Figure 2. Mean Percent Preferences for Concentrations

TABLE III

AOV: SIMPLE EFFECTS OF SPECIES BY CONCENTRATIONS INTERACTION

Source	df	SS	MS	F	P
Concentrations at quail	3	362.251	120.750	1.516	.75
Concentrations at gerbils	3	395.619	131.873	1.655	.75
Concentrations at squirrel monkeys	3	929.626	309.875	3.889	.05
Error (Within)	54	4,302.619	79.678		

TABLE IV

AOV: SIMPLE EFFECTS OF CHLORIDES BY CONCENTRATIONS INTERACTION

Source	df	SS	MS	F	P
Concentrations at NaCl	3	38.545	12.848	.161	.10
Concentrations at $SrCl_2$	3	867.722	289.241	3.630	.05
Concentrations at NH_4Cl	3	430.828	143.609	1.802	.75
Concentrations at KCl	3	214.974	71.658	.899	.50
Concentrations at $MgCl_2$	3	843.904	281.301	3.531	.05
Concentrations at $CaCl_2$	3	1,070.407	356.802	4.478	.01
Error (Within)	54	4,302.619	79.678		

as the large drop in mean percent preference shown by the squirrel monkeys with increasing concentrations. For the chlorides by concentrations interaction, the simple effects for concentrations at the $SrCl_2$ level were significant ($p<0.05$) as were the concentration simple effects at the $MgCl_2$ ($p<0.05$) and the $CaCl_2$ ($p<0.01$) levels. These significant simple effects can be seen in Figure 4 as the chlorides with the sharpest overall drop in mean percent preference with relation to increasing concentration.

Table V presents a set of t-tests which were performed on the differences between species mean percent preferences at each concentration level. All possible species comparisons were made at each concentration. At the 0.01M level, the mean percent preferences for the quail and gerbils were significantly ($p<0.01$) different from the mean percent preferences for the squirrel monkeys. At the 0.05M and 0.10M levels, the means for the quail and gerbils were significantly ($p<0.01$) different from each other as were the means for the squirrel monkeys and gerbils. No species means were significantly different at the 0.15 level. In Figure 3, it can be seen that at the 0.01M level the points corresponding to the mean percent preferences for the gerbils and quail are close together, but moderately separated from the mean for the squirrel monkeys. The means at the 0.05M level show the squirrel monkeys and quail to be close together, but both are widely separated from the mean for the gerbils. The same conditions hold true at the 0.10M level. At the 0.15M level, all three species' means are moderately close together.

In Table VI, a set of t-tests were made on the differences between mean percent preferences for the six chlorides at each concentration

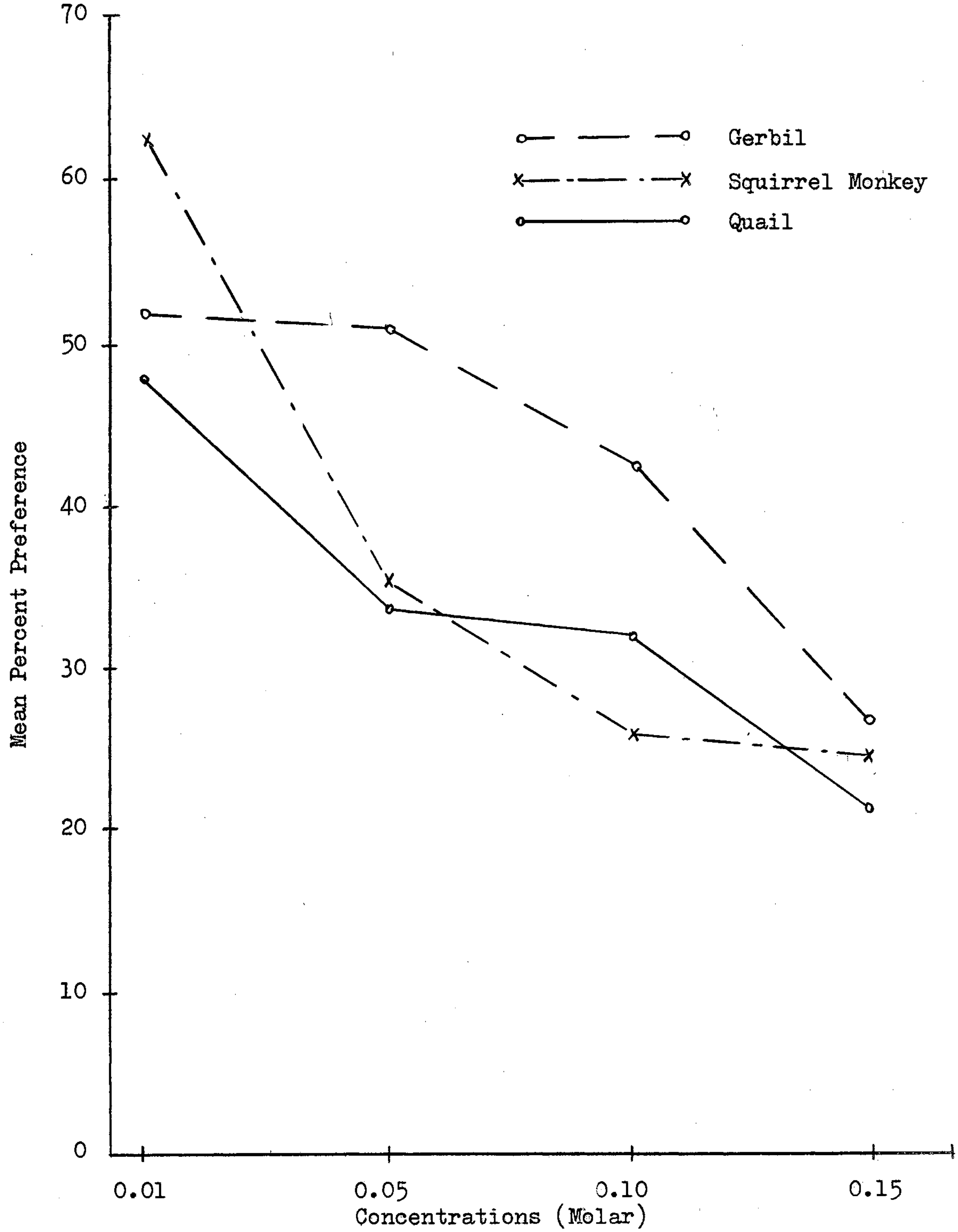

Figure 3. Species' Mean Percent Preferences over Concentrations

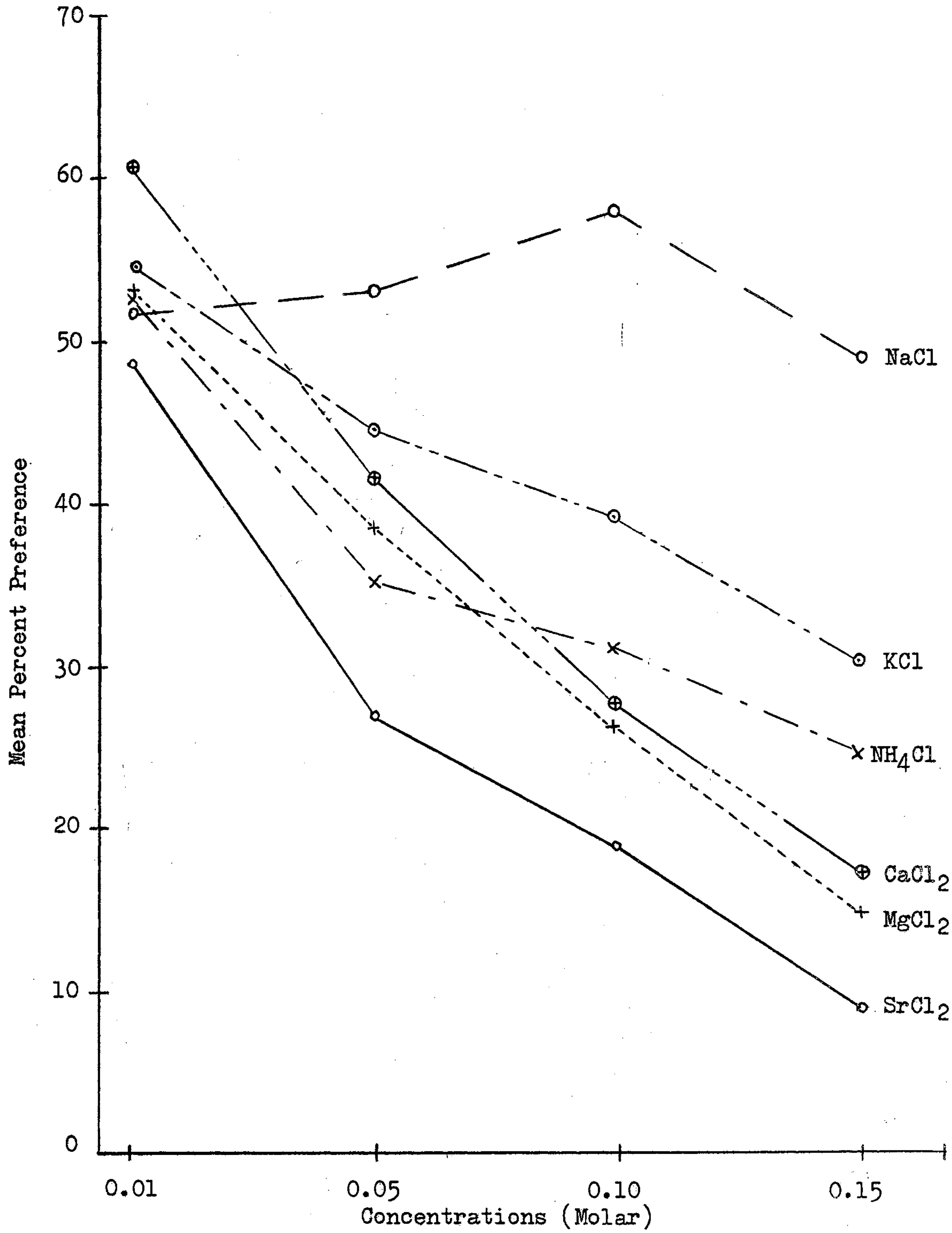

Figure 4. Mean Percent Preference for Chlorides over Concentrations

TABLE V

TESTS ON SPECIES MEANS AT EACH CONCENTRATION

	Means		
Concentration	Quail(1)	Gerbils(2)	Monkeys(3)
0.01	47.893	51.252	62.493
0.05	33.845	51.071	35.526
0.10	32.055	42.742	26.124
0.15	21.126	26.804	24.377

	Difference Between Means		
Concentration	(1)-(2)	(1)-(3)	(2)-(3)
0.01	- 3.359	-14.600*	-11.241*
0.05	-17.226*	- 1.681	+15.545*
0.10	-10.687*	+ 5.931	+16.618*
0.15	- 5.678	- 3.251	+ 2.427

Tests for difference between AC means: A at c_k

$S_{\bar{D}} = 6.114$

$t_{crit} = \pm 1.413$

$S_{\bar{D}}\ (t_{crit}) =$ Critical $\overline{AC}_{ik} - \overline{AC}_{i'k}$

$6.118\ (1.413) = \pm 8.639$

* Significant difference at $p<0.01$

TABLE VI

TESTS ON CHLORIDE MEANS AT EACH CONCENTRATION

Means						
	NaCl	$SrCl_2$	NH_4Cl	KCl	$MgCl_2$	$CaCl_2$
0.01M	52.218	48.674	52.760	54.904	53.832	60.889
0.05M	53.515	27.392	35.146	44.395	38.675	41.762
0.10M	58.023	19.037	31.403	39.057	26.675	27.849
0.15M	49.425	8.587	24.684	30.078	14.674	17.167

Difference Between Means					
	Na-Sr	Na-NH_4	Na-K	Na-Mg	Na-Ca
0.01M	+ 3.544	- 0.542	- 2.686	- 1.614	- 8.671
0.05M	+26.123*	+18.369*	+ 9.120	+14.840*	+11.753
0.10M	+38.986*	+26.620*	+18.966*	+31.348*	+30.174*
0.15M	+40.838*	+24.741*	+19.347*	+34.751*	+32.258*

	Sr-NH_4	Sr-K	Sr-Mg	Sr-Ca
0.01M	- 4.086	- 6.230	- 5.158	-12.215
0.05M	- 7.754	-17.003*	-11.283	-14.370*
0.10M	-12.366*	-20.020*	- 7.638	- 8.812
0.15M	-16.097*	-21.491*	- 6.087	- 8.580

	NH_4-K	NH_4-Mg	NH_4-Ca
0.01M	- 2.144	- 1.072	- 8.129
0.05M	- 9.249	- 3.529	- 6.616
0.10M	- 7.654	+ 4.728	+ 3.554
0.15M	- 5.394	+10.010	+ 7.517

	K-Mg	K-Ca
0.01M	+ 1.072	- 5.985
0.05M	+ 5.720	+ 2.633
0.10M	+12.382*	+11.208
0.15M	+15.404*	+12.208

	Mg-Ca
0.01M	- 7.057
0.05M	- 3.087
0.10M	- 1.174
0.15M	- 2.493

Tests for BC means: B at c_k

$S_{\bar{D}} = 8.647$

$t_{crit} = \pm 1.413$

$S_{\bar{D}} (t_{crit}) =$ Critical $BC_{jk} - BC_{j'k}$

$8.647 (1.413) = \pm 12.218$

* Significant difference at $p < 0.01$

level. At the 0.01M level, no significant difference between pairs of means was noted. At the 0.05M level, the means for $SrCl_2$, NH_4Cl, and $MgCl_2$ were significantly ($p<0.01$) different from NaCl, and the means for KCl and $CaCl_2$ were significantly ($p<0.01$) different from $SrCl_2$. At the 0.10M level, the means for $SrCl_2$, NH_4Cl, KCl, $MgCl_2$, and $CaCl_2$ were significantly ($p<0.01$) different from NaCl. At the same level, the means for NH_4Cl and KCl were significantly ($p<0.01$) different from $SrCl_2$, and the means for KCl and $MgCl_2$ were significantly ($p<0.01$) different from each other. At the 0.15M level, the same set of means were significantly ($p<0.01$) different as at the 0.10M level. In Figure 4, it can be seen that the majority of these significant differences stem from the fact that the NaCl curve does not drop with increasing concentrations as the curves for the other five chlorides do.

All differences between means were tested at the 0.01 significance level with a two-tailed t-test because, in creating the standard error of the difference between means, the pooling of heterogeneous sources of variation could produce a positive bias in the statistical tests of significance. Furthermore, making a large number of t-tests requires a high level of significance to decrease the probability of Type I errors. The critical "t" values at the 0.01 significance level were computed by a method recommended by Winer (1962, p. 324) when the standard error of the difference between means represents a pooling of possibly heterogeneous error terms in testing the difference between simple effect means in the repeated measures design.

The body weights of the quail at the end of preference testing ranged from 106 gms. to 158 gms. with a mean weight of 126 gms., S.D.= 15.8 gms. The body weights of the gerbils at the end of preference

TABLE VII

COMPARISON OF STIMULATIVE EFFICIENCIES OF CATIONS FOR THREE SPECIES

Species	Chlorides	Mean Percent Preference	Rank (Low to High)	Orders of Stimulative Efficiencies (High to Low)
Quail	NaCl	45.912	6	$Mg^{++} > Sr^{++} > Ca^{++} > NH_4^{+} > K^{+} > Na^{+}$
	$SrCl_2$	25.120	2	
	NH_4Cl	38.167	4	
	KCl	42.358	5	
	$MgCl_2$	25.058	1	
	$CaCl_2$	25.768	3	
Gerbils	NaCl	38.010	2	$Sr^{++} > Na^{+} > NH_4^{+} > Mg^{++} > K^{+} > Ca^{++}$
	$SrCl_2$	37.260	1	
	NH_4Cl	41.140	3	
	KCl	43.722	5	
	$MgCl_2$	42.692	4	
	$CaCl_2$	54.980	6	
Squirrel Monkeys	NaCl	75.980	6	$Sr^{++} > NH_4^{+} > Ca^{++} > Mg^{++} > K^{+} > Na^{+}$
	$SrCl_2$	15.387	1	
	NH_4Cl	28.687	2	
	KCl	40.246	5	
	$MgCl_2$	32.491	4	
	$CaCl_2$	30.006	3	

Order of Ionic Mobilities of Cations: $NH_4^{+} > K^{+} > Sr^{++} = Ca^{++} > Mg^{++} > Na^{+}$

testing ranged from 61 gms. to 93 gms. with a mean weight of 73 gms., S.D.=10.3 gms. The weights of the squirrel monkeys ranged from 429 gms. to 1070 gms. with a mean weight of 703 gms., S.D.=206.3 gms.

In Table VII, an analysis of the stimulative efficiencies of the cations as chlorides was made. In this study, the stimulative efficiency of a cation was defined in terms of the mean percent preference for a particular metallic chloride by a particular species across all the concentrations employed. If a particular species showed a high overall mean percent preference for a metallic chloride, then the cation of that chloride was assumed to have a low stimulative efficiency. In other words, it was assumed that the lower the stimulative efficiency of a cation as a chloride, the higher would be its overall mean percent preference by a particular species. The chlorides were ranked from most rejected (a low overall mean percent preference) to most preferred (a high overall mean percent preference). On the basis of the present definition of the stimulative efficiency of a cation, the order of ranking of the chlorides from most rejected to most preferred would produce an order of chlorides which would be the same as the order of stimulative efficiencies of the cations as chlorides. The results in Table VII show that none of the orders of stimulative efficiencies of the cations as chlorides appear to agree among the species tested, nor do they coincide with the order of ionic mobilities of the cations as defined by Frings (1946).

CHAPTER IV

DISCUSSION

Due to the fact that all factors in this study were assumed to be fixed, it is not appropriate to make broad generalizations about the results. Therefore, all conclusions and general statements apply only to the species, chlorides, and concentrations used in this study.

Little evidence was found to support the idea that species, by itself, is a significant factor in taste-preference behavior. This statement is based on the lack of significance for the species factor in the main analysis, but when species is considered in relation to another factor employed, its importance changes.

The species by concentrations interaction proved to be significant. This interaction means that there were significantly different reactions by each of the different species to the concentrations employed. At the 0.01M level, the squirrel monkeys were significantly different in their reactions from the gerbils and quail. At the 0.05M level and 0.10M levels, the squirrel monkeys and quail were similiar in their reactions, but they differed significantly from the gerbils. At the 0.15M level, all three species were similar in their reactions. Therefore, the impact of a species factor seems to lie in its interaction with the concentrations of the stimulus solutions employed.

The species by chlorides interaction was not significant, although the chloride factor was significant. This pattern of significance apparently means that the different types of chlorides had a major role in determining taste-preference behavior and that the role was similar in the species studied.

It should be noted that the chlorides by concentrations interaction was significant as were the chloride and the concentration main effects. The significance of the chlorides by concentrations interaction was mainly due to the rapid decline in preferences for $CaCl_2$, $MgCl_2$, and $SrCl_2$ with increasing concentrations while the preferences for NaCl, KCl, and NH_4Cl showed a slight or moderate decline. The concentration main effects showed a significant linear drop with increasing concentrations. The significance of the chloride main effects appeared to be due to the slight overall preference for NaCl; whereas the other chlorides were rejected.

These results cast new meaning on previous findings. The notion of "absolute species specific taste worlds" needs to be further clarified. In this study, it appears that the particular species, considered by itself, is not too important. What is important is the combination of species with other characteristics of the stimulus solutions. As shown in this study, the particular metallic chloride with which the stimulus solution was made does not seem to interact with the species factor, but the concentration of the stimulus solution does interact with the species factor. All of this points to the importance of considering not just single factors by themselves, but to considering the various individual factors in combination. Too much importance has been placed on individual factors as major determiners of taste-preference behavior. Although analytical emphasis has been placed on individual factors, it has been typical of many investigators to examine their data in the form of interactions. With regard to this last point, it should be noted that most investigators have viewed their results as presented in Figures 5, 6, and 7. These figures represent the species by chlorides by concentrations interaction. It appears from Figures 5, 6, and 7 that a significant species variable is operating. For example, in Figure 5 the squirrel monkeys show an increasing preference for NaCl with increasing concentrations, but in Figures 6 and 7 the gerbils and quail show a decreasing preference for NaCl with increasing concentrations. A second example can be seen in Fugure 7 where it appears that the quail have combined the chlorides into two groups, one moderately rejected and the other highly rejected. This grouping is not apparent in Figures 5 and 6 for the gerbils and squirrel monkeys. These examples tend to show what appears to be a significant species by chlorides by concentrations interaction, but the statistical analysis indicates otherwise. All this points to the fact that although most investigators in taste-preference behavior have viewed their results as interactions, they have failed to analyze them as interactions. Instead, they have analyzed their data in terms of individual factors. Future attempts at explaining taste-preference behavior should be cognizant of unique effects due to factors in combination.

Previous reports of agreement between the stimulative efficiencies of cations as chlorides and the order of cation mobilities receive little support from this study. There was little agreement between the orders of stimulative efficiencies of the cations as chlorides and the cation mobilities as defined by Frings (1946) for the species tested. This lack of agreement could be due to the fact that a different method of determining the order of stimulative efficiencies of the cations was

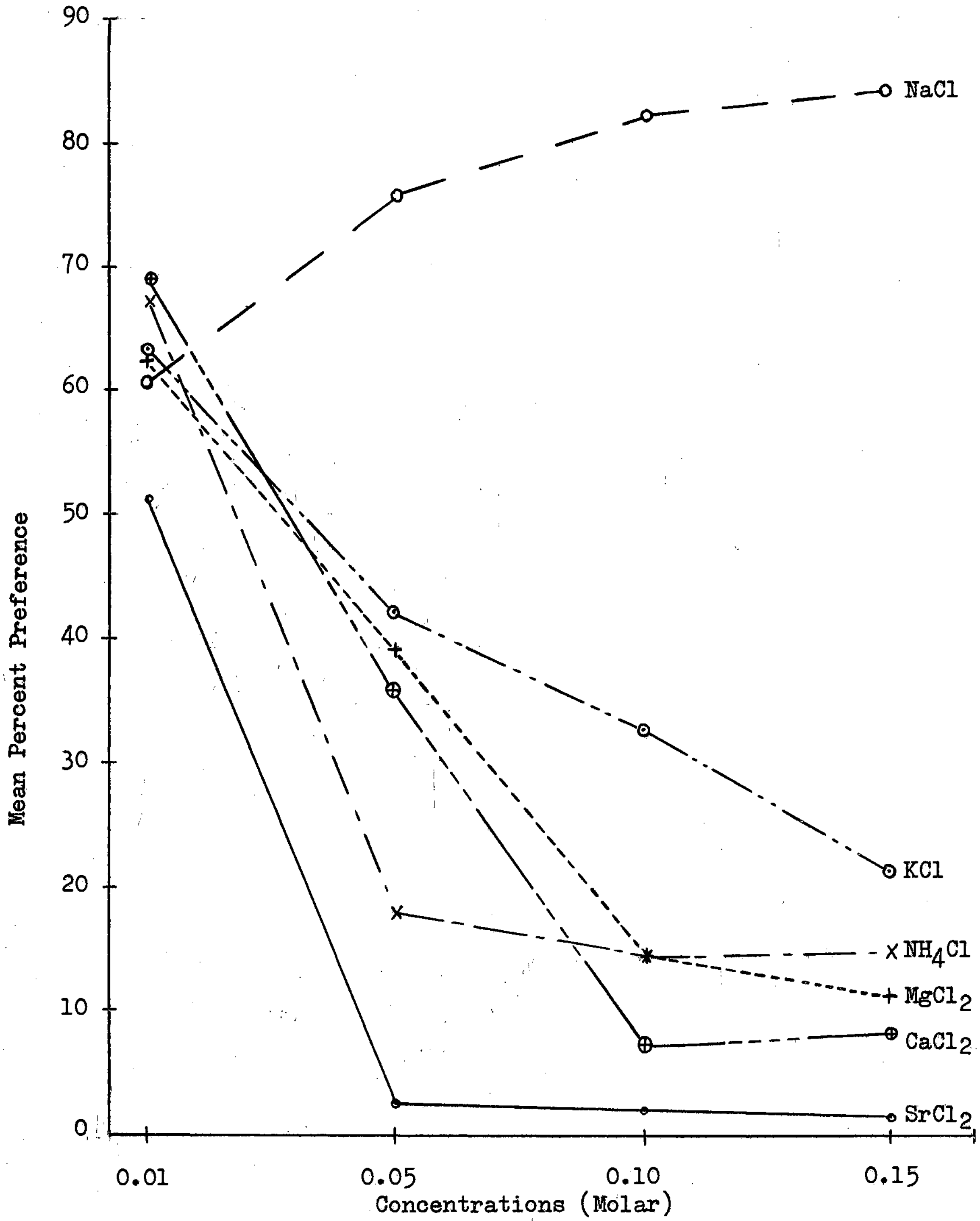

Figure 5. Mean Percent Preferences by Squirrel Monkeys for Chlorides over Concentrations

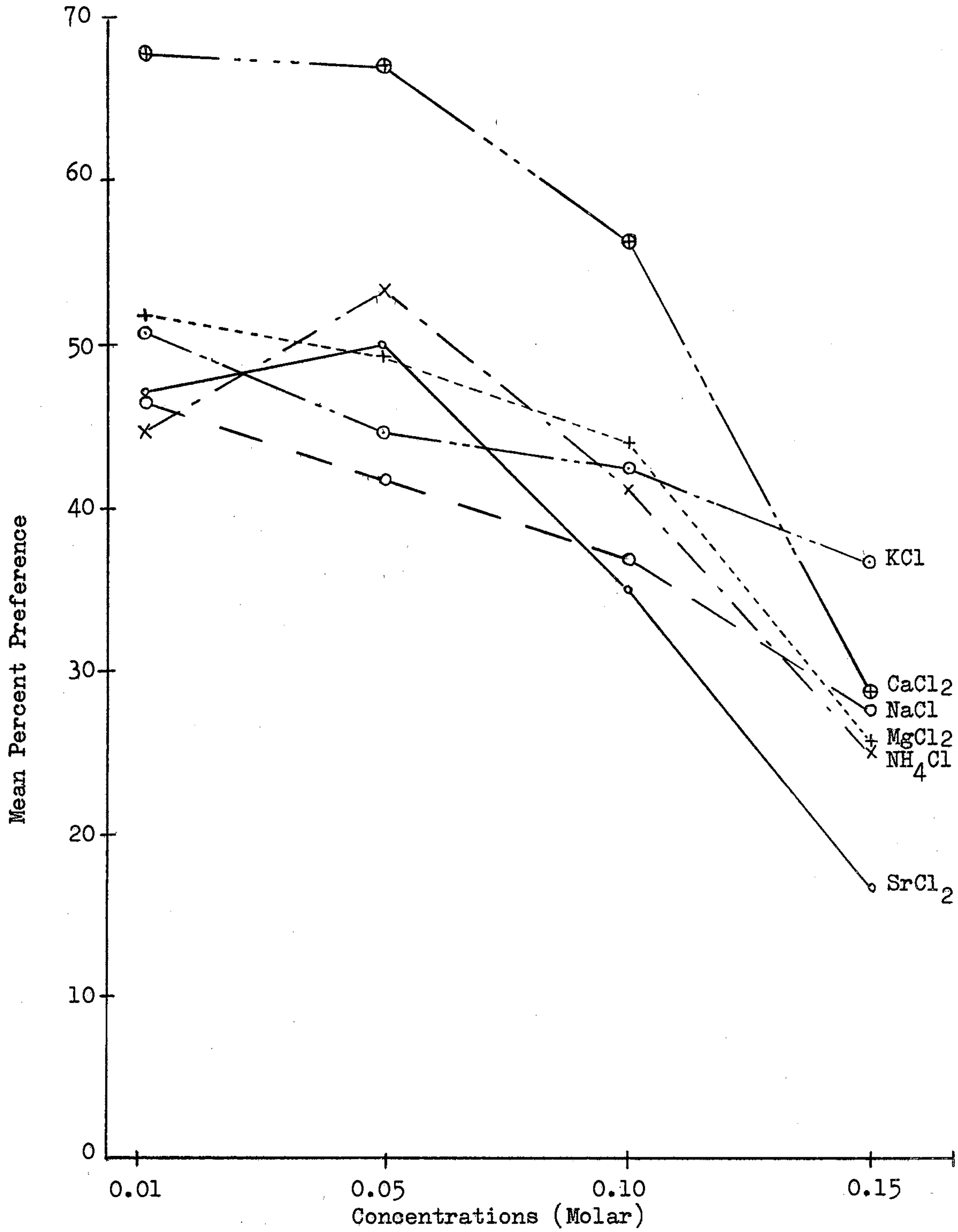

Figure 6. Mean Percent Preferences by Gerbils for Chlorides over Concentrations

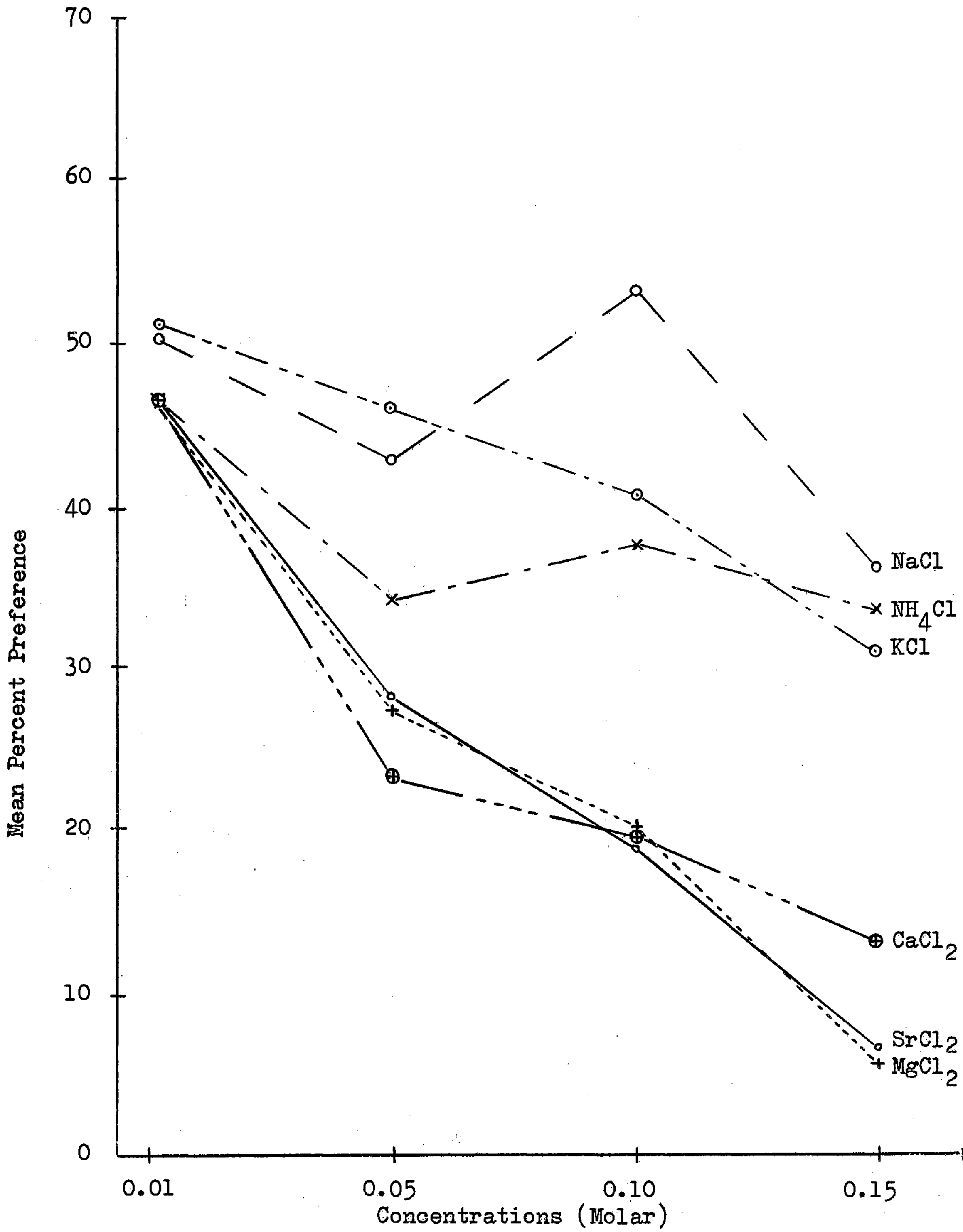

Figure 7. Mean Percent Preference by Quail for Chlorides over Concentrations

used in this study than in previous studies. The mean percent preference values over the four concentration levels were used, and the chlorides were ranked from most rejected to most preferred on the basis of these overall mean percent preference values. The stimulative efficiency of each cation was defined in terms of the overall mean percent preference by a particular species for a chloride, with the cation of that chloride ranked high in terms of stimulative efficiency if the overall mean percent preference for the chloride was low.

This study also provides little support for a similarity of the orders of stimulative efficiencies of cations as chlorides across species. This fact seems to indicate that the species are somehow different in their taste-preference behavior. Actually, these orders reflect the non-significant species by chlorides by concentrations interaction. Whether or not the orders of stimulative efficiencies of the cations were significantly different across species is questionable.

The results of this study have cast doubt on both the notion of "absolute species specific taste worlds" and on the notion that there is a similarity among species in terms of taste-preference behavior based on the orders of stimulative efficiencies of cations as chlorides. This study has presented evidence of a species-to-species similarity in taste-preference behavior with respect to the types of metallic chlorides employed. There was also evidence for a unique reaction among the species studied to concentrations of chlorides. It is probable that whatever similarity there is among species, it is not based on the stimulative efficiencies of the cations as chlorides. This latter conclusion may not be valid. It should be noted that the orders of stimulative efficiencies of the cations were defined in an operationally different way from that used by Frings.

The statistical design in the present study is subject to two major criticisms. First, the small number of subjects (n=2) in each experimental unit did not allow for a good estimation of error variability and could have resulted in effects which were unique to a pair of individuals and not characteristic of the species. Second, the repeated measures design has a differential sensitivity, with the between subjects effects being tested with less sensitivity than the within subjects effects. Thus, the effects due to species, chlorides, and species by chlorides interaction were tested with less sensitivity than concentrations and interactions with concentrations.

One possible solution to the differential sensitivity problem would be the use of a complete factorial design without repeated measures on concentrations. However, this would lead to problems of obtaining enough subjects and of testing them. There are a number of other suggestions for future research resulting from this study. The investigation could be repeated using a complete factorial design with a reduction in the number of levels of the chlorides factor. The levels could be reduced to NaCl, $SrCl_2$, and KCl. These three chlorides were found to encompass most of the reactions to the other three chlorides. Another readily apparent suggestion is that of repeating the basic plan of this investigation, but changing the chlorides factor to sweet-tasting compounds. Finally, it would be desirable to investigate different species besides those used in this study. In this way, a body of knowledge could be developed on taste-preference behavior.

CHAPTER V

SUMMARY

This investigation was concerned with the taste-preference behavior of Japanese quail (*Coturnix coturnix japonica*), Mongolian gerbils (*Gerbillus gerbillus*), and squirrel monkeys (*Saimiri sciureus*) to six metallic chlorides (NaCl, $SrCl_2$, KCl, $MgCl_2$, NH_4Cl, and $CaCl_2$) at four molar concentrations (0.01, 0.05, 0.10, and 0.15M). The species were selected so as to span a fairly broad range of the phylogenetic series. The chlorides were selected on the basis of the facts that they are colorless in solution with water and are nonpoisonous at hypotonic and isotonic concentrations. The concentration levels were chosen so as to minimize the contribution of postingestive factors and to maximize the oral stimulation factors in taste-preference behavior.

A review of literature revealed only a small number of studies which could be classified as comparative studies of taste-preference behavior. These studies were contradictory concerning the similarity or dissimilarity of taste-preference behavior across species. One general notion found was that of "absolute species specific taste world" (Kare, and Ficken, 1963). An opposite notion concerning a degree of similarity among species was also found (Frings, 1946, 1951). Since neither concept was explicitly stated, it was not possible to develop specific, testable hypotheses. Instead, it was decided that it would be better to perform an empirical investigation and then collate the results with the two previously mentioned notions.

The basic plan of the investigation was that of a three-factor, factorial design with repeated measures on one factor (molar concentrations). There were 12 subjects selected from each species for a total of 36 subjects. The subjects in each species were randomly paired and each pair was randomly assigned to one of the six metallic chlorides. Each subject in a pair received a two-bottle preference test in which one fluid container held the appropriate chloride solution and the other distilled water. The two-bottle preference test lasted six days at one concentration. During this period, readings were taken every 24 hours on the amount of chloride solution and distilled water consumed by each subject. The two fluid containers were reversed in position at the time of reading. The total chloride solution intake over the six-day period and the total distilled water intake over the same period of time were summated to give a total fluid intake value for each subject. The total chloride solution intake over the six-day period was divided by the corresponding total fluid intake to give a ratio which was multiplied by 100 to produce a percent preference value for each subject. Each subject in a pair received four concentrations of the appropriate chloride solution in ascending order of concentration for a total of 24 days of preference testing.

The results indicated that the species factor was not significant, but the species by concentrations interaction was significant ($p < 0.05$). This was interpreted as meaning that the species employed showed an overall similarity in a taste-preference behavior, but that the behavior in relation to a particular concentration was unique to the species. The species by chlorides interaction and the species by chlorides by concentrations interaction were not significant.

The concentration factor was significant ($p<0.001$), as was the chloride factor ($p<0.05$). The chlorides by concentrations interaction was significant ($p<0.05$), and was interpreted as meaning that the effect of the various chlorides on taste-preference behavior differed as a function of concentrations. All chlorides resulted in a decreasing mean percent preference value with increasing concentrations, and $SrCl_2$, $MgCl_2$, and $CaCl_2$ showed the greatest rejection.

Orders of stimulative efficiencies of the cations as chlorides were defined in terms of the overall mean percent preference value for each chloride by each species. There was no apparent agreement among the orders of stimulative efficiencies for the different species, or with the order of cation mobilities as defined by Frings (1946).

It was concluded that the notion of "species specific taste worlds" probably needs modification. The crucial factor is probably not species per sec, but the unique way different species respond to particular aspects of stimulus solutions. It was noted that each of the species produced a different order of stimulative efficiencies of the cations, but it was doubted that these orders were statistically significant aspects of taste-preference behavior.

Criticisms of the study were noted, and disadvantages of the repeated measures design were mentioned. Problems for further research were suggested, including the replication of this study with different taste stimuli and/or species.

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Master of Science

Thesis: A COMPARATIVE STUDY OF THE TASTE REACTIONS OF JAPANESE QUAIL, MONGOLIAN GERBILS, AND SQUIRREL MONKEYS TO SIX METALLIC CHLORIDES

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