

ROLE OF INGESTION IN AVERSION LEARNING

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BY

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ABSTRACT

The role of ingestion in taste and odor aversion learning is usually considered to be limited to bringing subjects in contact with the gustatory and olfactory conditioned stimuli. Results of the present series of experiments indicate that ingestion not only facilitates contact with certain cues, but under certain circumstances, also facilitates the conditioned aversion behavior. Rats that drink a novel-flavored substance prior to toxicosis evidence stronger learned aversions to the taste in subsequent drinking preference tests than subjects that experience the novel flavored substance in the absence of ingestive behaviors during conditioning. Similarly, ingestion during odor-aversion learning results in stronger aversion behavior, provided subjects drink the same solution during postconditioning tests as had been ingested during conditioning. Control groups demonstrated that the facilitory effect of ingestion on odor-toxicosis learning is not a result of acquired aversions to the flavor of the solution ingested. Rather, the results are consistent with the hypothesis that subjects drinking during odor-toxicosis conditioning acquire aversion not only to the CS odor but also to additional cues arising from an interaction of the CS odor and the flavor of the ingested solution. Additional evidence suggests that this interaction probably occurs in the central nervous system and not at the level of the receptors.

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TABLE OF CONTENTS

	Page
CHAPTER 1: INTRODUCTION.....	1
CHAPTER 2: ROLE OF INGESTION IN TASTE-AVERSION LEARNING	17
CHAPTER 3: ROLE OF INGESTION IN ODOR-TOXICOSIS LEARNING.....	58
CHAPTER 4: CONCLUSION.....	113
REFERENCES.....	

FIGURES

- Figure 1. Experiment 1: Saccharin preference scores for the post-conditioning test session. Experiment 1a: Subjects conditioned during curarization with either toxic or control injections following saccharin (Groups cS-T and cS- \bar{T}) or either toxic or control injections preceding saccharin (Groups cT-S and c \bar{T} -S). Experiment 1b: Subjects conditioned in the noncurarized, normal state with either toxic or control injections following ingestion of saccharin (Groups nS-T and nS- \bar{T}) or either toxic or control injections preceding ingestion of saccharin (Groups nT-S and n \bar{T} -S).
- Figure 2. Experiment 2: Amount ingested and percentage of time drinking during conditioning in Experiment 2 for subjects ingesting saccharin from a drinking tube (Group FI) or receiving a fast oral infusion of saccharin (Groups WF-Li and DF-Li) or a slow oral infusion of saccharin (Group DS-Li). Except for Group WF-Li, all subjects were water deprived during conditioning.
- Figure 3. Experiment 2: Median of the mean percent saccharin preference of poisoned (Li) and control (Na) subjects for the two postconditioning test sessions in Experiment 2. During conditioning, poisoned subjects ingested the saccharin from a drinking tube (Group FI) or received either a fast oral infusion of saccharin (Groups WF-Li and DF-Li) or a slow oral infusion of saccharin (Group DS-Li). All poisoned subjects except Group WF-Li were water deprived during conditioning.

- Figure 4. Experiment 3: Saccharin preference scores of subjects conditioned either while 23 1/2 hr water deprived (Groups D-Li and D-Na) or after access to 20 ml of water (Groups W-Li and W-Na). Groups D-Li and W-Li received lithium injections during conditioning, while Groups D-Na and W-Na received saline injections.
- Figure 5. Experiment 4: Saccharin and buzzer-water preferences of subjects exposed to both saccharin and the buzzer in conditioning with lithium, shock, or saline.
- Figure 6. Experiment 5: (A) Saccharin preferences of subjects exposed to saccharin in conditioning with lithium, shock, or saline. (B) Buzzer-water preferences of subjects exposed to the buzzer in conditioning with lithium, shock, or saline.
- Figure 7. Experiment 6: Cumulative water intakes of Ingestion and No-Ingestion poisoned (Li) and control (Na) subjects in the presence of the odor of Mentholatum after the first conditioning trial in Experiment 1.
- Figure 8. Experiment 6: Cumulative water intakes of Ingestion and No-Ingestion poisoned (Li) and control (Na) subjects in the presence of the odor of Mentholatum after the second conditioning trial in Experiment 1.
- Figure 9. Experiment 7: Cumulative water intakes in the presence of the odor of Mentholatum after the second conditioning trial for Ingestion and No-Ingestion poisoned subjects conditioned without the Mentholatum odor.

- Figure 10. Experiment 8: Cumulative water intakes in the presence and absence of the odor of Mentholatum for Ingestion and No-ingestion poisoned subjects conditioned with the Mentholatum odor.
- Figure 11. Experiment 9: Top view of odor-choice apparatus. (Arrows indicate exhaust fans; see text for further details.)
- Figure 12. Experiment 9: Mean percent preference for the Mentholatum odor for poisoned Ingestion and No-Ingestion subjects as well as saline controls during tests without fluids (Test 1: numbers above bars indicate mean total sec spent exploring both test compartments. For Tests 2 and 3, numbers above bars indicate mean total ml ingested in the test compartments.)
- Figure 15. Experiment 9: Mean cumulative time spent exploring the Mentholatum test compartment during Test 1 (no fluids available) for Ingestion and No-Ingestion poisoned subjects as well as saline controls.
- Figure 14. Experiment 10: Mean percent preference for the Mentholatum odor for poisoned Ingestion and No-Ingestion subjects as well as saline controls during tests without fluids (Test 1) and with saccharin and water (Tests 2 and 3). (For Test 1, numbers above bars indicate mean total sec spent exploring both test compartments. For Tests 2 and 3, numbers above bars indicate mean total ml ingested in the test compartments.)
- Figure 15. Experiment 11: Saccharin preference scores of normal and peripherally anosmic subjects after taste-toxicosis

Figure 15. conditioning.
(cont'd)

Figure 16. Experiment 13: Mean percent preference for the Mentholatum odor for anosmic (a) and normal (b) Ingestion and No-Ingestion subjects as well as saline controls during tests without fluids and with water. (For no-fluid tests numbers above bars indicate mean total sec spent exploring both tests compartments. For tests with water numbers indicate mean total ml ingested in the test compartments.)

CHAPTER 1: INTRODUCTION

Poisons play an important role in the organization of plant and animal life. Although non-living matter can be poisonous, many of the poisons that are of ecological importance are "secondary substances" synthesized by plants to govern their relationship to other plants and animals (Whittaker & Feeny, 1971). Certain of the higher plants, for example, synthesize poisons which inhibit the germination and growth of seeds of other higher plants which would otherwise provide competition for space and mineral resources. Others produce substances toxic to animals.

Since plants are the basic food source for the animal kingdom, the presence of poisons in plants has provided evolutionary pressure for the development of mechanisms that protect animals against toxins. Some animals have evolved mechanisms for detoxifying certain poisons. Others sequester the poisonous substances so as not to experience the toxic effects. Detoxification and sequestration mechanisms provide animals with access to poisonous food avoided by other animals. In certain cases, the presence of specific poison has even come to act as an attractant which guides animals with detoxifying or sequestering mechanisms to the poisonous plant (Whittaker & Feeny, 1971).

Although sequestering poisons achieves some of the same effects as the detoxifying mechanisms, sequestration of poisons has the additional advantage that animals with sequestered poisons are not only unaffected by the poison but also become unpalatable to their predators. For example, monarch butterflies readily feed on milkweed containing cardiac glycoside poisons which do not affect them but are toxic to blue jays that feed on

the monarchs (Brower, Brower, & Corvino, 1967).

In addition to detoxification and sequestration mechanisms that protect animals from the effects of poisons, certain animals have evolved a learning mechanism whereby they come to avoid eating substances that have made them sick on previous occasions. Of the various modes of minimizing poisoning, psychologists are most interested in this learning mechanism of poison avoidance.

Current psychological research on poison avoidance learning developed from several applied research programs started as a result of problems encountered during World War II. Two of these programs involved attempts to develop effective rodent control techniques in the United States and the United Kingdom. In the course of this work it soon became apparent that rats avoid poisoned bait if they experienced postingestional illness after eating such bait on a previous occasion (Richter, 1953; Rzoska, 1953). Thus, successful rodent population control through the use of poisoned foods requires poisoned bait which kills rats the first time it is eaten. A third research program involved investigating the behavioral effects of exposure to ionizing radiation. The results of greatest interest to psychologists were obtained with irradiation used as an unconditioned stimulus in a learning paradigm. Rats learned aversions to the taste of a relatively novel saccharin solution, for example, when ingestion of the saccharin was followed by exposure to gamma radiation (Garcia, Kimeldorf, & Koelling, 1955).

The two rodent control programs did not stimulate much systematic scholarly research on poison-avoidance behavior, although they formed the basis of a rather interesting agricultural application in which poison

avoidance behavior in mice was shown to be of potential benefit to farmers of Douglas fir in northern California (Tevis, 1956). Once Douglas fir is logged in certain areas of northern California, the region becomes invaded by tan oak because conditions are not favorable for the growth of conifers. Attempts to seed denuded areas with Douglas fir often fail because forest mice consume the seeds. Extermination of the mice is not effective because non-poisoned mice from neighboring areas quickly invade the denuded territory. The problem can be solved, however, by adding a non-lethal poison to the Douglas fir seeds so that mice learn to avoid eating Douglas fir seeds without being killed or leaving the area (Tevis, 1956).

In contrast to the rodent control programs, early demonstrations that rats acquire taste aversions as a result of experiencing the taste before exposure to ionizing radiation have stimulated much further research. This work was at first pursued primarily by John Garcia and his colleagues but has recently attracted the interest of many other investigators.

Some Early Experiments

Garcia's initial efforts to explore the avoidance of stimuli associated with ionizing radiation were directed at demonstrating that such avoidance learning is similar to other learning effects studied in psychological laboratories. In one of the early experiments, for example, Garcia and Kimeldorf (1957) investigated the importance of the temporal relationship between drinking saccharin and exposure to gamma radiation. Four groups of rats were exposed to irradiation for four hours while another group served as a nonirradiated control. The manipulation of primary interest was the availability of a highly palatable saccharin solution in relation to radiation exposure. One group had the saccharin

available for two hours just preceding the four-hour exposure period. Other groups had the saccharin available during either the first or last two hours of irradiation, while the last irradiated group had saccharin available for two hours just after irradiation. These treatments are analogous to trace, simultaneous, and backward conditioning sequences often tested in classical conditioning (Kimble, 1961, pp. 47-48.) Consistent with results in most classical conditioning situations, Garcia and Kimeldorf found that groups having the saccharin solution available during exposure to irradiation (simultaneous conditioning) learned stronger aversions to saccharin than subjects that had saccharin available just before irradiation (trace conditioning), although the trace conditioning group also learned an aversion to saccharin. In contrast, subjects having the saccharin solution available just after irradiation (backward conditioning) failed to learn a taste aversion.

In another early study Garcia, Kimeldorf, and Hunt (1957) attempted to demonstrate that, as in other learning preparations, a variety of stimuli can be used as the to-be-conditioned stimulus with ionizing radiation as the unconditioned stimulus. Specifically, they showed that rats can learn aversions to spatial cues experienced in conjunction with gamma or X irradiation. The nature of the spatial cues responded to, whether visual, olfactory, or tactile, however, was not ascertained.

These two experiments appeared to support the notion that radiation-induced aversions were similar to other forms of associative learning. Subsequent research showed that cues of different modalities were not equally associable with toxicosis (Garcia, Kimeldorf, & Hunt, 1961). However, such CS modality effects were also not inconsistent with

observations in other learning preparations.

Long-delay Learning

In contrast to Garcia and his colleagues, several other investigators expressed doubts about a learning interpretation of radiation-induced aversions. McLaurin and Scarborough (1963) pointed out that the trace conditioning procedures used by Garcia and his colleagues to condition taste aversions with ionizing radiation may have allowed delays between exposure to the taste and subsequent irradiation on the order of several minutes, delays which, on the basis of work in other learning preparations, would not be expected to support associative learning (Kimble, 1961, pp. 155-160). For example, in the Garcia and Kimeldorf (1957) experiment, subjects in the trace conditioning group received access to saccharin for two hours prior to irradiation. No data concerning temporal drinking patterns during the two-hour period is presented. However, since subjects were water deprived, they probably drank to satiation long before the end of the two-hour period, thus introducing a delay between taste and subsequent irradiation. Troubled by this possibility, McLaurin and Scarborough (1963) sought to determine the effects of various delays between exposure to a taste and subsequent irradiation. If radiation-induced taste aversions are in fact a form of associative learning, progressive delays between exposure to a taste and subsequent irradiation should result in progressive decrements in taste-aversion learning. The saccharin aversion learning of rats exposed to X-irradiation 0, 25, or 50 minutes after a 10-minute period of access to saccharin was assessed in saccharin-water preference tests conducted soon after conditioning. Contrary to predictions from a learning interpretation, progressive delays

between exposure to saccharin and X-irradiation did not weaken the taste aversions observed. Even the group exposed to X-irradiation 50 minutes after saccharin, an interval unprecedented for usual demonstrations of associative learning, acquired a strong aversion to saccharin.

McLaurin (1964) repeated the McLaurin-Scarborough experiment with groups of rats exposed to X-irradiation 3, 60, 120, and 180 minutes after access to saccharin. The saccharin preference of subjects was tested immediately after irradiation. As in the previous experiment, all irradiated subjects acquired aversions to saccharin with no differences among the 60-, 120-, and 180-minute delay groups. Introducing delays between the taste and X-irradiation again did not have the expected decremental effect on taste aversion learning. Further evidence against a learning interpretation of the radiation-induced taste-aversion effect was obtained in a group which had not been given saccharin prior to X-irradiation but also evidenced an aversion to saccharin during the post-irradiation test.

In contrast to the findings of McLaurin and Scarborough, numerous other investigators have found orderly decrements in taste aversion learning as a function of the delay between access to a flavor and subsequent toxicosis. In the first such demonstration Garcia, Ervin, and Koelling (1966) used apomorphine hydrochloride injections (7 mg/kg, i.p.) as the US instead of X-irradiation and compared groups of rats injected with the toxin 30, 45, 75, 120 and 180 minutes after access to saccharin. Five conditioning trials were conducted, and subjects were tested for their response to saccharin three days after the last conditioning trial. Increasing the delay between access to saccharin and the apomorphine injections during

conditioning attenuated the learning of saccharin aversions. And, no aversions were learned in groups where the apomorphine injections were delayed 120 or 180 minutes.

The basic findings of Garcia, Ervin, and Koelling (1966) that delays between taste and toxicosis result in orderly decrements in taste aversion learning have since been replicated by numerous other investigators (Kalat & Rozin, 1971; Nachman, 1970; Revusky, 1968; Smith & Roll, 1967; Wright, Foshee, & McLeary, 1971). Furthermore, the difference in results between these experiments and the experiments of McLaurin and Scarborough cannot be explained in terms of the unconditioned stimulus used, since two of these studies also used X-irradiation (Revusky, 1968; Smith & Roll, 1967).

The inconsistency between the McLaurin and Scarborough experiments and subsequent studies showing that delays between taste and toxicosis result in decrements in taste aversion acquisition were resolved by a series of experiments showing that rats can learn aversions to flavors experienced immediately after exposure to X-irradiation, presumably because the aversive effects of radiation last for a considerable time after radiation exposure (McLaurin, Scarborough, & Farley, 1964; Morris & Smith, 1964; Scarborough, Whaley, & Rogers, 1964; Smith, Taylor, Morris, & Hendricks, 1965; see also review by Smith, 1971). In the McLaurin and Scarborough experiments all subjects were tested for saccharin aversions soon after exposure to X-irradiation. Thus, groups with various delays between taste and toxicosis, as well as subjects not given saccharin before irradiation, had equal opportunity to associate saccharin given during the postexposure test with the prolonged aversive aftereffects of irradiation.

This aversion learning motivated by the aversive aftereffects of X-rays was probably responsible for lack of differences among groups. The other studies of the effects of delay between taste and toxicosis avoided this technical problem by testing for taste aversions at least twenty-four hours after toxicosis, by which time the aversive aftereffects of the irradiation or other toxins used were presumably no longer present.

Consistent with a learning interpretation of toxicosis-induced taste aversions, it is now well established that increasing the delay between taste and toxicosis results in weaker taste aversions, subjects nevertheless acquire aversions with delays between taste and poisoning of more than an hour. In the first successful delay experiment, rats learned taste aversions even if the taste-toxicosis interval was 75 minutes (Garcia, et al., 1966). Revusky (1968) was able to induce taste aversions with a 6.5-hour interval between taste and X-irradiation. In previous learning experiments little associative learning was observed if the CS preceded the US by more than a few seconds (Kimble, 1961, pp. 155-160). In the taste aversion paradigm delays on the order of several hours between CS (taste) and US (toxicosis) do not appear to preclude their association.

One obvious way to reconcile long-delay learning in the taste aversion paradigm with the requirement of a close temporal relationship in other examples of associative learning is to assume that somehow the taste of the flavored substance used stays in the mouth during the delay interval and is therefore present when the animal is made sick. However, much evidence contrary to this aftertaste hypothesis has been obtained (for a review see Revusky & Garcia, 1970). It is unlikely that after-tastes persist for 6.5 hours, an interval which does not preclude aversion

learning (Revusky, 1968), and even if there were an aftertaste 6.5 hours after ingesting a flavored solution, the aftertaste might be different from the original flavor.

The most convincing data that aftertastes probably do not mediate the delay interval are provided by experiments in which subjects ingest a second flavor between the CS taste and toxicosis. Garcia, Green, and McGowan (1969), for example, were able to condition an aversion to a .05% hydrochloric acid solution with a 60-minute delay interval even though the rats ate an average of 9 grams of dry food during the delay and tests with litmus paper did not reveal any acid on the tongue 2 minutes after subjects drank the HCl solution. Similarly, Kalat and Rozin (1971) showed that rats could learn an aversion to sucrose with a 30-minute delay to poisoning even if they were allowed to drink novel salt and coffee solutions during the delay. (When subject were allowed to drink three differently-flavored solutions during the delay interval they still developed an aversion to the sucrose. However, tests of significance were based on the amount of sucrose ingested in a postconditioning sucrose-water choice test. Since there were differences in total fluid intake between conditioned and control groups, the two groups probably were not significantly different in preference for sucrose.)

The fact that long-delay learning of taste-aversions cannot easily be explained by a peripheral-aftertaste hypothesis suggests that some central mechanism is mediating the CS-US delay interval. Central mediation of CS-US delay intervals on the order of several hours has not been previously described in experiments on associative learning. Thus, while toxicosis-induced taste aversions appear to involve associative learning,

the nature of this learning seems quite different from the kinds of learning previously investigated by psychologists.

Specificity of Cue to Consequence

Just as further investigations of the temporal relationship between taste and ionizing radiation led to an unexpected result, namely that very long delays between taste and toxicosis are tolerated in taste aversion learning, further investigations of aversion learning to nongustatory cues in the rat also yielded unanticipated effects. Garcia, et al. (1957) reported that rats can associate spatial cues with ionizing radiation, but that spatial aversion learning requires more conditioning trials and higher radiation intensities than taste aversion learning (Garcia, et al., 1961). Consistent with this observation, Rozin (1969) found that the introduction of a half-hour delay between CS and US greatly interferes with the association of nongustatory cues with toxicosis but does not preclude taste aversion learning.

In contrast to the successful demonstrations of spatial aversion learning motivated by toxicosis described above, spatial aversions are typically not found in poison avoidance learning experiments. Several investigators have found that while rats learn to avoid the taste of poisoned bait, they do not learn to avoid the location of the poisoned bait (Hargrave & Bolles, 1971; Rozin, 1967; Tevis, 1956). In these experiments it may be that the taste of the poisoned bait overshadowed (Kamin, 1969) the cues provided by location. However, attempts to condition aversions to auditory, spatial, and visual cues in the absence of concurrently available distinctive gustatory stimuli using toxicosis have also provided negative results (Domjan & Wilson, 1972; Garcia & Koelling,

1967; Garcia, McGowan, Ervin, & Koelling, 1968).

The fact that nongustatory cues do not become associated with toxicosis as well as novel taste stimuli is a result not unexpected on the basis of findings from other learning experiments. Differences in CS effectiveness with a given US are often observed. Kamin, for example, found better conditioning with a light than with a 50 db noise in the conditioned suppression situation (Kamin, 1969). Increases in stimulation are also often more effective as CSs than decreases (e.g., Gormezano, 1972; Kamin, 1965).

Perhaps gustatory stimuli are more effective in aversion learning than spatial or audiovisual cues because rats perceive taste stimuli as more "salient" or more intense in some sense. Alternatively, perhaps the aversion learning preparation draws the attention of the rats away from spatial or audiovisual cues and towards the taste stimuli. Such explanations suggest that in aversion learning paradigms, taste cues would be more effective than spatial or audiovisual stimuli regardless of the nature of the aversive unconditioned stimulus used. To assess this hypothesis, Garcia and Koelling (1966) allowed rats to drink a distinctively flavored solution from a drinking tube that also produced distinctive audiovisual stimulation whenever the rats made contact with it. Some subjects were poisoned after experiencing the taste-audiovisual complex while others were exposed to painful electric foot-shock. As in other experiments, postconditioning tests showed that poisoned subjects acquired aversions to the gustatory stimulus but did not learn to avoid the audiovisual cue. Contrary to expectation, however, shocked subjects avoided the audiovisual cue but not the taste stimulus. This specificity

of cue to consequence has since been replicated by both Garcia and his colleagues (Garcia, et al., 1968) as well as Domjan and Wilson (1972), and shows that the differential effectiveness of gustatory and non-gustatory cues in aversion learning cannot be explained in terms such as CS intensity, salience, or distinctiveness which do not make reference to the unconditioned stimulus used.

Explanations

Research on poison avoidance learning therefore led to the discovery of two phenomena inconsistent with traditional assumptions concerning the mechanism of associative learning: the long-delay learning effect and the specificity of cue to consequence effect. These phenomena have, in turn, stimulated reformulations of the theoretical mechanisms assumed to operate in associative learning.

The first such "reformulation" was offered in a paper by Garcia and Ervin (1968) in which they proposed that animals evolve specific receptor systems that govern the types of stimuli that they can learn to associate readily. According to the hypothesis the long-delay learning effect and the specificity of cue to consequence effect are results of the evolution of neural structures comprising a gustatory-visceral system that facilitates the integration of afferents from gustatory and visceral receptors and a telereceptor-cutaneous system that facilitates the integration of afferents from audiovisual and cutaneous receptors.

The proposal of two specific receptor systems accounts for the specificity of cue to consequence effect by assuming that associations between CSs and USs within the same receptor system are made more easily than associations between different receptor systems. The hypothesis is

also supported by much neuroanatomical evidence (see Garcia & Ervin, 1968, for details). However, it fails to explain satisfactorily why long-delay learning is possible in the gustatory-visceral system but is not possible nearly to the same degree in the telereceptor-cutaneous system. Garcia and Ervin suggest that the integration of afferents in the telereceptor-cutaneous system occurs at a higher level in the nervous system than the integration of afferents in the gustatory-visceral system and that integration at higher levels may require closer temporal relationships. This suggestion, though, is admittedly speculative.

Other reformulated conceptions of associative learning proposed after Garcia and Ervin (1968) are similar to the original suggestion in assuming that animals acquire specialized learning mechanisms through evolution but are far less precise about the specific neurological adaptations (Rozin & Kalat, 1971; Seligman, 1970). Because of this lack of precision one cannot predict what kinds of associations animals will be able to form. By considering the environmental contingencies with which animals have to cope, various learning mechanisms that would be adaptive can be designed, but one cannot predict which of the various possible adaptations will actually evolve. Furthermore, to justify the claim that a particular learning mechanism is adaptive, more than just the "reasonableness" of the suggestion is required. Only a study of the reproductive success of animals with and without the presumed adaptive learning mechanism can conclusively demonstrate that the mechanism in question is in fact adaptive. Such demonstrations, however, have not been conducted for any of the presumed adaptive learning mechanisms. Thus, the idea that learning mechanisms are adaptive lacks predictive

power and is supported only by speculation. In contrast, the Garcia-Ervin hypothesis in which associative learning is a function of the degree of neural integration between associated stimuli allows predictions about learning derived from neurology and, in the case of the specificity of cue to consequence effect in rats, is supported by the neurological evidence reviewed by Garcia and Ervin (1968). Hypotheses stating that learning mechanisms are the result of natural selection, however, do provide an adaptive-evolutionary context in which to view learning--a framework previously not explicitly considered by many learning psychologists.

Both the Garcia-Ervin and the adaptive-learning-mechanisms hypotheses were formulated largely in response to the discovery that learning about taste cues apparently is different from learning about audiovisual cues. This difference in the learning mechanisms involved with taste and audiovisual cues conceptually could be a result of any of the various differences between gustatory and audiovisual stimulation. Both the Garcia-Ervin and the adaptation hypotheses attribute the difference in learning about taste and audiovisual cues to the fact that usually the two types of stimuli inform the organism about different aspects of the environment. The Garcia-Ervin hypothesis also emphasizes the fact that taste and audiovisual cues stimulate different receptors in the organism and therefore "enter" the nervous system via different afferent pathways. There is, however, yet another major difference between taste and audiovisual stimulation. In most learning situations audiovisual cues are typically imposed on the subject despite or in spite of the subject's behavior. In order to receive the stimulation the subject does not have

to make particular orientation movements or be motivated to come in contact with the stimulation. Furthermore, receipt of the stimulation does not result in complex physiological processes which continue after the stimulus has been perceived. In contrast, novel taste stimulation is usually experienced only if the subject is motivated to ingest the vehicle carrying the taste cue and approaches, orients towards, and eats the source of the taste. Furthermore, reception of the taste stimulus in this way results in complex digestive activities. Therefore, differences in learning about taste and audiovisual cues may be a result of differences in the way in which rats come in contact with these stimuli.

Only one major theory of aversion learning has specifically called attention to differences in the reception of taste and audiovisual stimulation (Revusky, 1971). Unlike the Garcia-Ervin and the adaptive-specialization hypotheses, this theory was formulated to explain only the long-delay learning effect in taste aversions and does not account for the specificity of cue to consequence effect. In fact, the theory uses the latter effect to explain the former. It states that since novel tastes are encountered only as a consequence of ingestion and only tastes are easily associated with toxicosis, rats can learn taste aversions with long delays between taste and toxicosis because they do not encounter many associable cues during the delay interval. In contrast, since audiovisual cues are perceived in the absence of specific approach and orientations behaviors and are readily associated with foot shock, rats cannot learn audiovisual aversions with long delays between audiovisual stimulation and foot shock because they encounter

many associable cues during the delay interval.

Although the Revusky interference theory notes that taste and audiovisual cues reach the organism differently, this difference is not of importance to the formal structure of the theory. The theory does not say that tastes are associated with toxicosis over long delays because the reception of taste stimuli requires approach and ingestive behaviors. The presence or absence of associable cues during the delay interval carry the main burden of explaining the presence or absence of long-delay learning.

Since the reception of taste and audiovisual stimulation involves such markedly different mechanisms, however, it is conceivable that these differences are in part responsible for differences in learning with gustatory and audiovisual cues. The experiments described in the present thesis were therefore designed to elucidate the role of ingestive and approach behaviors in aversion learning. Two series of experiments were conducted, one using the taste-aversion learning paradigm (see Chapter 2) and the other using the odor-aversion learning paradigm (see Chapter 3). The results of both series of studies confirmed that ingestive behaviors are an important variable in aversion learning.

CHAPTER 2: ROLE OF INGESTION IN TASTE-AVERSION LEARNING

The experiments reported in the present chapter were designed to investigate the contribution of orientation, approach, and ingestive behaviors to taste-toxicosis learning. Gustatory stimulation was provided by a 0.2% sodium saccharin solution, which is normally highly preferred over water by rats. Toxicosis was provided by sublethal intraperitoneal injections of 0.12 M lithium chloride. Results of the taste-toxicosis conditioning trials were assessed in choice tests with both saccharin and water simultaneously available because choice tests appear to be particularly sensitive to taste-aversions (Dragoin, McClearly, & McCleary, 1971). (Although Grote and Brown (1971) also concluded that choice tests of taste aversions are more sensitive than single stimulus tests, their comparison was confounded with differences in time since poisoning.)

Experiment 1: Aversion learning curarized rats

To study the contribution of approach and ingestive behaviors to taste aversion learning, the aversion learning of subjects making contact with a gustatory stimulus in conjunction with ingestion during conditioning has to be compared to the aversion learning of subjects experiencing the gustatory cue in the absence of ingestive behaviors. Various techniques have been used that permit the placement of flavored solutions directly into the oral cavity of rats in the absence of orientation and approach behaviors. (Gross, Trapold, & Hyder, 1968; Kissileff, 1969; Phillips & Norgren, 1970). These techniques, however, still permit subjects to engage in ingestive behaviors once the flavored solutions have been introduced in the oral cavity. In the present

experiment subjects were paralyzed by curare to restrain them from engaging in ingestive behaviors in response to flavored solutions introduced into the oral cavity during taste-toxicosis conditioning. The curare paralysis also insured that the taste stimuli would be experienced in the absence of unconditioned postingestional stimulation because the flavored solution flowed out of the mouth in the absence of ingestive behaviors. The effects of isolating taste from ingestion and post-ingestional stimulation during taste-toxicosis conditioning were assessed by comparing the taste-aversion learning of curarized subjects with the aversion learning of noncurarized rats poisoned after freely ingesting the CS flavor.

Method

Experiment 1a.-- Twenty-three male Sprague-Dawley rats (Holtzman; Madison, Wisconsin), 250 to 300 gm, were individually housed with continual access to food and 1-hr daily access to water. Eleven subjects were anesthetized with ether, and a cannula consisting of a small-diameter polyethylene tube (Clay-Adams, "intramedic," P.E. 205) was inserted in the cheek through a hole made by a #12 hypodermic needle. (The two ends of the cannula were flared and held in place by polyethylene washers.) Four days after cannulation, these subjects were conditioned while paralyzed for 1 1/2 to 3 hr by an intraperitoneal injection of d-tubocurarine chloride (1.2 mg/kg, Squibb) and artificially respired using an E & M Physiograph respirator set at 70 breaths/min, I:E ratio of 1:1, and pressure = 20 cm of water, to maintain normal blood-gas parameters (see Dicara, 1970). During conditioning, a 0.2% sodium saccharin solution (weight/volume in tap water) was presented

for 2 min by manually infusing 10 ml directly into the oral cavity with a blunted needle fitted into each subject's cheek cannula. (Recovery of the infused solution running out of the mouth showed that none was swallowed.) Immediately after the saccharin presentation, six subjects were injected intraperitoneally with 6 ml of 0.12 M lithium chloride, a toxin which in this dose produces occasional diarrhea and mild ataxia lasting 45 to 60 min in noncurarized rats. These six subjects comprised the curarized (c), saccharin-toxin (S-T) group (Group cS-T). The remaining five subjects were given control injections of 6 ml of isotonic sodium chloride instead of the toxin: Group cS- \bar{T} (curarized, saccharin-no toxin).

To assess the importance of giving the US injections after presentation of the flavored solution, the 12 remaining subjects were injected with the toxin ($n=6$) or sodium chloride ($n=6$) at the beginning of the curarization period and had 10 ml of saccharin dripped on their tongues from the blunted tip of a needle for 2 min 1 1/2 to 3 hr later when they started recovering from the paralysis (as evidenced by the return of skeletal movement) but were still unable to ingest the saccharin solution. These subjects, comprising the curarized toxin-saccharin (cT-S) and curarized no toxin-saccharin (c \bar{T} -S) groups, were sufficiently recovered from the paralysis to breathe without artificial respiration 5 to 90 min (median = 35 min) after the saccharin presentation.

After recovery from the curare-induced paralysis, subjects received access to tap water for 1 hr. The next day, after 23 hr of water deprivation, subjects had access to both the saccharin solution and tap water for 1 hr in the home cage. Saccharin preference was

measured as the percentage of total fluid intake consisting of saccharin.

Experiment 1b. -- In order to compare the aversion learning of subjects conditioned in the curarized state with the aversion learning of subjects freely ingesting the CS solution, four additional groups were used in tests paralleling those of Experiment 1a. Twenty-four male Sprague-Dawley rats (Holtzman; Madison, Wisconsin), individually housed with continual access to food and 1-hr daily access to water, were never curarized and were allowed to drink 10 ml of saccharin in the home cage during conditioning. One group ($n=6$) was injected with 6 ml of the toxin after ingesting the saccharin (Group nS-T: normal, saccharin - toxin), while another group ($n=6$) was injected with 6 ml of isotonic sodium chloride instead (Group nS-T̄). Two additional groups ($n=6,6$) were injected with either the toxin or saline 1 1/2 to 3 hr before ingesting the 10 ml of saccharin (Groups nT-S and nT̄-S, respectively). (Subjects in Groups nT-S and nT̄-S were yoked with subjects in Groups cT-S and cT̄-S of Experiment 1a to determine the injection-to-saccharin intervals.) Postconditioning test procedures were identical to those of Experiment 1a. All two-group comparisons were made with the Mann-Whitney U test (two-tailed).

Results and Discussion

Experiment 1a. -- Saccharin preference scores from the postconditioning test session are shown in the left panel of Figure 1. Subjects injected with the toxin after exposure to saccharin during conditioning (Group cS-T) had lower saccharin preferences than each of the three control groups (Groups cS-T̄, cT-S, and cT̄-S; all $p < .02$), all of which had comparably high preferences. No group differences in total fluid intake during the preference test were observed.

Figure 1

Experiment 1: Saccharin preference scores for the post-conditioning test session. Experiment 1a: Subjects conditioned during curarization with either toxic or control injections following saccharin (Groups cS-T and cS- \bar{T}) or either toxic or control injections preceding saccharin (Groups cT-S and c \bar{T} -S). Experiment 1b: Subjects conditioned in the noncurarized, normal state with either toxic or control injections following ingestion of saccharin (Groups nS-T and nS- \bar{T}) or either toxic or control injections preceding ingestion of saccharin (Groups nT-S and n \bar{T} -S).

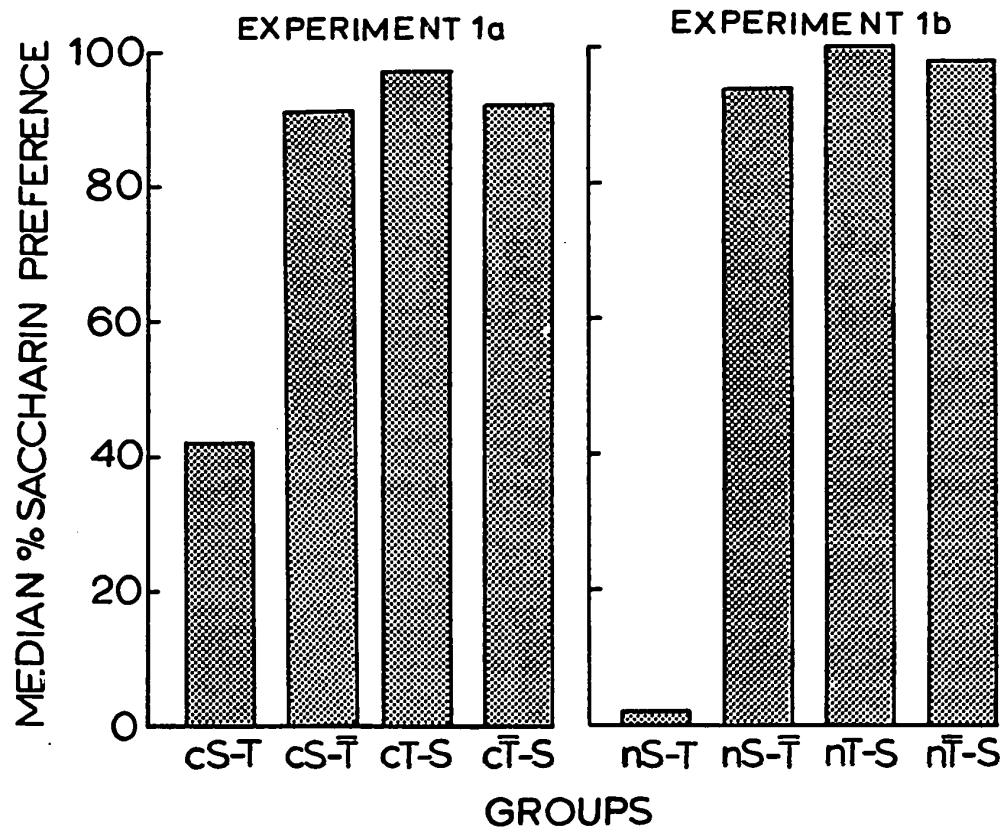


Figure 1

The curare technique used in Experiment 1a insured that during aversion conditioning subjects experienced the CS flavor in the absence of both ingestive responses and unconditioned postingestional stimulation. The fact that subjects poisoned after tasting saccharin formed stronger aversions than saline-injected controls, or subjects poisoned before experiencing saccharin, shows that ingestion and postingestional stimulation are not necessary for taste-aversion learning. Rats can learn an aversion to a flavor by merely tasting the flavored substance prior to toxicosis. This finding is consistent with a recent report showing that rats can learn taste-aversions in the absence of ingestion if the CS flavor is presented by intravenous infusion during conditioning (Bradley & Mistretta, 1971).

Experiment 1b.-- No group differences in total fluid intake during the preference test were observed. Saccharin preference scores of subjects in Experiment 1b are displayed in the right panel of Figure 1. In contrast to Experiment 1a, subjects in Experiment 1b ingested the saccharin solution during conditioning while in the noncurarized, normal state. Those that were injected with the toxin after drinking saccharin (Group nS-T) showed a markedly low saccharin preference in comparison with the three control groups (Groups nS- \bar{T} , nT-S, and n \bar{T} -S; all $p_s < .01$), all of which evidenced comparably high preferences.

The depression in saccharin preferences shown by subjects injected with the toxin after drinking saccharin in the normal state (Group nS-T, Experiment 1b) were much more pronounced than the saccharin aversions of subjects poisoned after tasting, but not ingesting, the saccharin while curarized (Group cS-T, Experiment 1a; $p < .01$). In

contrast, the high saccharin preferences of control subjects conditioned in the normal state (Groups nS- \bar{T} , nT-S, and n \bar{T} -S, Experiment 1b) were comparable to those of corresponding controls conditioned while curarized (Groups cS- \bar{T} , cT-S, and c \bar{T} -S, Experiment 1a).

The fact that subjects conditioned while curarized formed weaker aversions than subjects permitted to approach and ingest the saccharin flavor during training suggests that ingestion facilitates the learning of taste aversions. The difference in aversion learning obtained between curarized and normal subjects is, however, open to several other interpretations. For example, gustatory aversion learning under curare may not completely transfer to the normal state. Or, the copious salivation of curarized subjects (Miller, 1969; Smith, Brown, Toman, & Goodman, 1947) may dilute and alter the saccharin flavor. Thus, for subjects conditioned under curare, the taste of the conditioning solution might have been different from the taste of the test solution, and the weaker saccharin aversions found might have resulted from a generalization decrement.

Experiment 2: Aversion learning in the absence of
ingestive behaviors in nonparalyzed rats.

Experiment 1 did not permit an unambiguous assessment of the contribution of ingestion to taste-aversion learning because the variation in extent of ingestion between curarized and freely moving subjects was confounded with differences in immobility, excessive salivation, and other aspects of the difference between the curarized and normal states. The purpose of Experiment 2 was to explore further the possible contribution of ingestive behaviors to aversion learning

while minimizing the artifacts introduced by the curarization technique. A cannula preparation was used permitting infusion of flavored solutions directly into the oral cavities of nonparalyzed rats. Preliminary work showed that water-satiated subjects tended to ingest less of the infused solution than deprived rats, and, if a sufficiently rapid infusion speed was used (46 ml/min), satiated subjects did not ingest any of the infused solution. Thus, manipulations of infusion speed in combination with the level of water deprivation permitted systematic variations in ingestion of the CS solution.

Method

Subjects and Preexperimental Preparation.-- Forty-eight male Sprague-Dawley rats, 350 to 400 gm (Canadian Breeding Farms; Quebec, Canada), were individually housed with food always available and access to water limited to 25 min each day. After 3 days on the maintenance schedule, a cannula identical to that used with Groups cS-T and cS-T̄ of Experiment 1a was fitted into the cheek of each subject.

Adaptation.-- To expedite recovery of infused fluids not swallowed, subjects were restrained in a slightly inflated blood pressure cuff and held manually as necessary above a funnel attached to a graduated cylinder whenever solutions were infused into the oral cavity. Five days of adaptation to the restraining procedure were conducted after the cannula implantation. On the first day, each subject was wrapped in the blood pressure cuff several times for a few seconds. On each of the next 4 days, subjects were restrained in the pressure cuff for 6 min.

During the following three daily sessions, subjects had tap water infused into the oral cavity for 2 min while in the restraint. The water was infused with an infusion pump (Harvard Model 941) at either a fast (F) or a slow (S) speed: 46 ml/min or 3 ml/min.

Sixteen subjects received all adaptation sessions just after their daily 25 min of water (W), while the remaining 32 subjects received the adaptation sessions while 23 1/2 hr deprived (D), just before the 25 min daily water periods. The fast infusion speed was used with the 16 watered subjects (WF) and 16 of the deprived subjects (DF), while the remaining 16 subjects had the water infused slowly (DS). Thus, 16 subjects were assigned to each of three adaptation conditions: watered-fast (WF), deprived-fast (DF), or deprived-slow (DS).

Conditioning.-- Aversion conditioning, the day after the last adaptation session, consisted of a 0.7% body weight intraperitoneal injection of either 0.12 M lithium chloride or isotonic sodium chloride 25 min after 2 min of contact with a 0.2% sodium saccharin solution. (A smaller dose of lithium was used than in Experiment 1 to avoid the complete depression of saccharin preference seen in Group nS-T and thus permit observation of differences in aversion learning.)

Nine subjects from each of the three adaptation conditions (watered-fast, deprived-fast, and deprived-slow) were injected with lithium chloride (Li) after having the saccharin infused into the oral cavity via the cheek cannula. For each subject, the level of deprivation and the infusion speed used during conditioning were the same as during the adaptation sessions. These subjects constituted Groups WF-Li, DF-Li, and DS-Li ($n=9,9,9$).

Four subjects from each of the three infusion adaptation conditions were treated as subjects in Groups WF-Li, DF-Li, and DS-Li except for receiving injections of isotonic sodium chloride instead of lithium after the oral infusions of saccharin. (The data for one subject in the deprived-fast condition was lost because of a procedural error.)

The remaining nine subjects, three from each adaptation condition, were allowed to ingest the saccharin solution freely from a drinking tube for 2 min and were injected with lithium 25 min later. These subjects constituted Group FI (free-ingestion).

Subjects receiving exposure to saccharin while 23 1/2 hr water deprived were allowed access to water for 25 min immediately after the saccharin presentation to insure all subjects equal opportunity for ingesting fluids before the toxin or control injections.

The amount of saccharin ingested during conditioning was measured for each subject. (For subjects receiving the saccharin by oral infusion, the amount not swallowed was recovered and subtracted from the amount infused.) In addition, an independent observer, unaware of the group-assignment of the subjects, recorded the percentage of time subjects receiving saccharin by oral infusion were observed making jaw movements indicative of drinking.

Testing.-- On the day after aversion conditioning, preference for saccharin versus water was measured in a 30 min preference test while subjects were 23 1/2 hr water deprived. Two graduated drinking tubes, one containing water and the other saccharin, were clipped to the front of the home cages. The position of the two tubes was reversed

for a similar test on the second day. Preference was measured as the percentage of the total liquid intake consisting of saccharin.

All two-group comparisons were made with the Mann-Whitney U test (two-tailed).

Results

Conditioning. -- The various experimental groups drank different amounts of the CS solution during conditioning. Figure 2 shows both the amount of saccharin ingested during conditioning and the percentage of time subjects were observed making jaw movements indicative of drinking for each lithium-injected group. Subjects freely ingesting the saccharin solution in the home cage (Group FI) as well as those getting the saccharin infused slowly into the oral cavity while 23 1/2 hr water deprived (Group DS-Li) had median intakes of at least 4.5 ml of saccharin and were observed making drinking movements more than 98% of the time. In contrast, deprived subjects receiving a fast oral infusion of saccharin (Group DF-Li) drank a median of only 2.0 ml and made drinking movements 53% of the time during the saccharin presentation. The difference between Group DF-Li and each of the Groups FI and DS-Li was significant for both response measures (all $p_s < .02$).

Watered subjects receiving a fast oral infusion of saccharin (Group WF-Li) did not ingest any of the saccharin and were observed drinking only 1% of the time. Subjects in Group WF-Li were significantly different from each of the other three lithium-injected groups in both amount of saccharin ingested and percentage of time observed drinking (all $p_s < .01$).

There were no significant differences in total fluid intake

Figure 2

Experiment 2: Amount ingested and percentage of time drinking during conditioning in Experiment 2 for subjects ingesting saccharin from a drinking tube (Group FI) or receiving a fast oral infusion of saccharin (Groups WF-Li and DF-Li) or a slow oral infusion of saccharin (Group DS-Li). Except for Group WF-Li, all subjects were water deprived during conditioning.

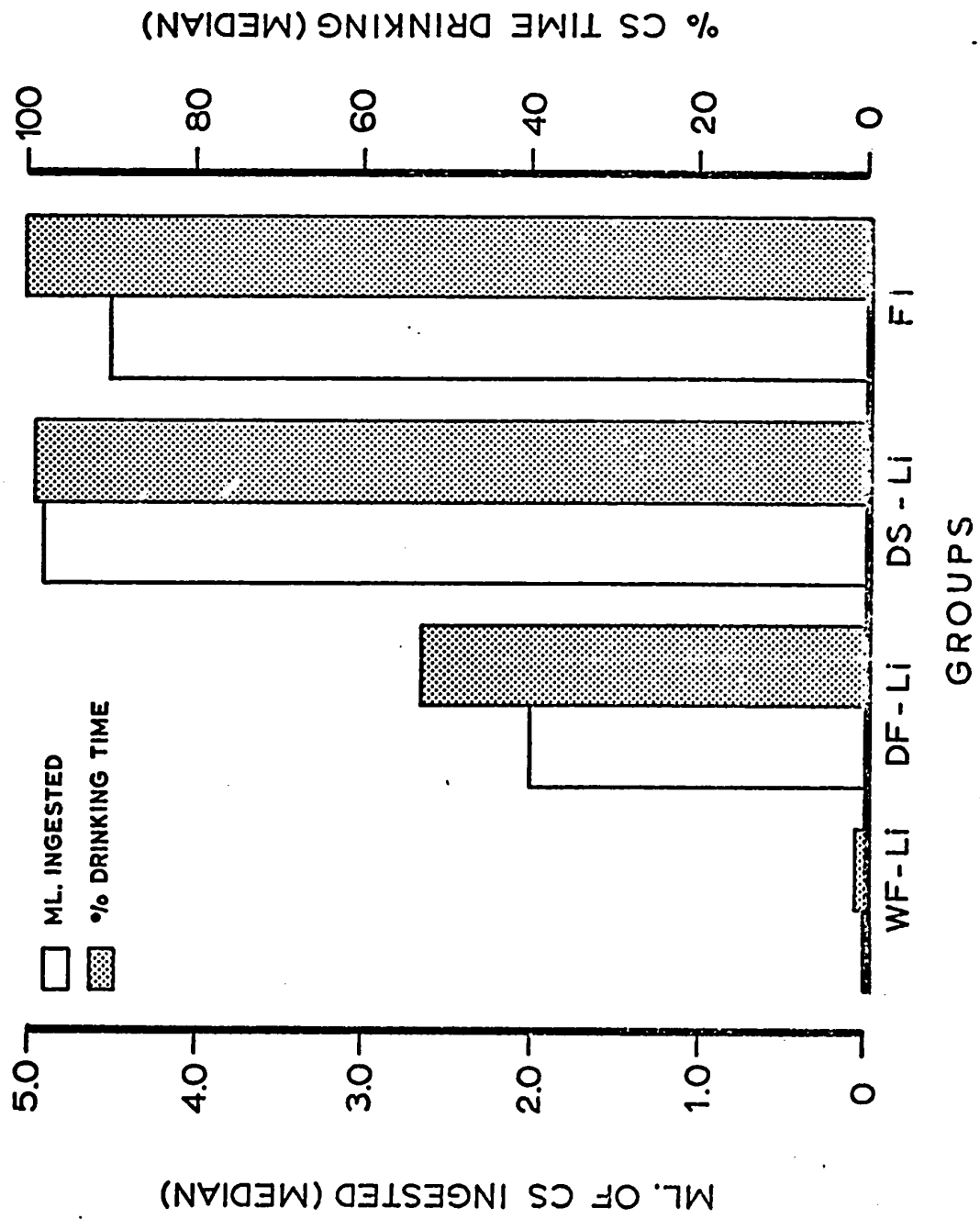


Figure 2

(saccharin + water) prior to the lithium injections on the conditioning day.

Testing.--The results of the postconditioning preference tests are shown in Figure 3. Groups injected with isotonic sodium chloride during conditioning were combined to form Group Na since no significant differences were found among them. Each group of lithium-injected subjects formed stronger aversions to saccharin than sodium-injected controls (all $p_s < .01$). Subjects that failed to ingest any of the saccharin solution during conditioning (Group WF-Li) formed weaker aversions to saccharin than each of the other lithium-injected groups (all $p_s < .02$), all of which showed comparably low saccharin preferences. No reliable differences in total fluid intake during the test sessions were found.

Discussion

The results of the present experiment are consistent with the hypothesis that ingestive responses facilitate taste aversion learning. Subjects that failed to ingest any of the CS during conditioning (Group WF-Li) acquired weaker aversions to saccharin than groups that drank at least 2 ml of the saccharin solution before poisoning (Groups FI, DS-Li, and DF-Li).

Only complete elimination of ingestive responses during conditioning resulted in an attenuation of taste aversion learning. A reduction in the amount of the CS solution ingested during conditioning from 4.5 to 2.0 ml had no appreciable effect on subsequent aversions. Thus, it seems that the facilitory effect of ingestion on taste-aversion learning does not depend on the consumption of very much of the CS solution.

Figure 3

Experiment 2: Median of the mean percent saccharin preference of poisoned (Li) and control (Na) subjects for the two postconditioning test sessions in Experiment 2. During conditioning, poisoned subjects ingested the saccharin from a drinking tube (Group FI) or received either a fast oral infusion of saccharin (Groups WF-Li and DF-Li) or a slow oral infusion of saccharin (Group DS-Li). All poisoned subjects except Group WF-Li were water deprived during conditioning.

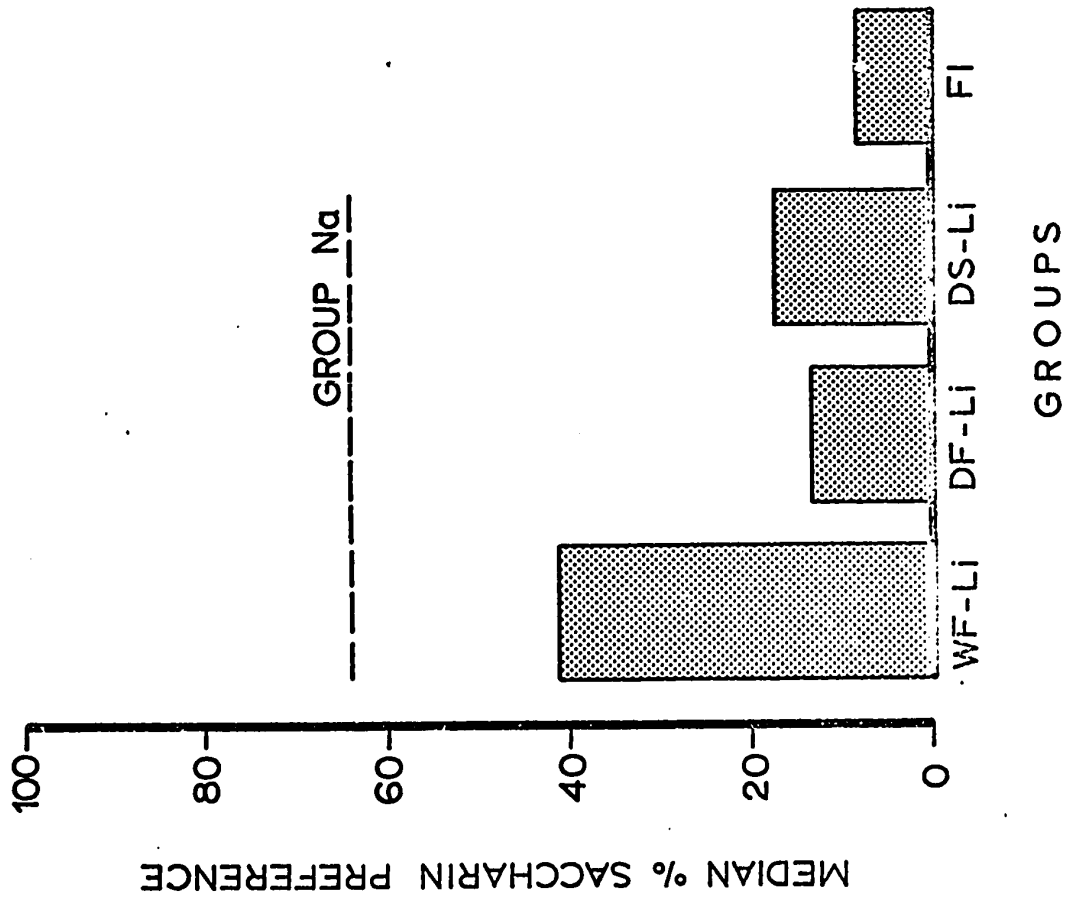


Figure 3

The results of the present experiment also suggest that behaviors involved in orienting towards and approaching the drinking tube do not facilitate taste aversion learning. Group DS-Li, which drank as much during conditioning as subjects drinking from a drinking tube (Group FI) but received the saccharin via the oral fistula in the absence of orientation and approach behaviors, acquired as strong saccharin aversions as Group FI.

The attenuated aversion learning of subjects experiencing saccharin in the absence of ingestive behaviors during conditioning probably did **not** result from the restraint used since Groups DF-Li and DS-Li, which did not evidence attenuated aversions, were also restrained in the blood pressure cuff during conditioning. Furthermore, restrained subjects receiving a slow oral infusion of saccharin (Group DS-Li) drank as much of the saccharin during training as subjects permitted to ingest the saccharin in the home cage (Group FI).

The speed used to infuse the saccharin solution into the oral cavity of subjects in Group WF-Li during conditioning also cannot account for their weak taste aversions since the same infusion speed was used with subjects in Group DF-Li, where no decrement in aversion learning was observed.

Although the attenuated aversion learning of subjects in Group WF-Li cannot be attributed to either the restraint or the infusion speed used, it should be noted that Group WF-Li was conditioned just after receiving the daily ration of water whereas all other groups were conditioned while 23 1/2 hr water deprived. Thus, the weakened aversions observed in Group WF-Li may have resulted from experiencing the saccharin

flavor in a reduced drive state during conditioning, rather than from the suppression of ingestive responses.

Experiment 3: Aversion learning in watered and deprived rats.

To see if the weaker taste aversions observed in subjects experiencing the CS flavor in the absence of ingestive behaviors in Experiment 2 were a result of the fact that these subjects were less thirsty during conditioning than each of the other poisoned groups, Experiment 3 was designed to investigate the possible effects of a reduction in drive state on aversion learning while minimizing accompanying changes in ingestive behavior. To encourage ingestion in the absence of thirst, solutions were infused very slowly into the oral cavity of freely moving rats.

Method

Subjects and Preexperimental Preparation. -- Thirty-seven male Sprague-Sawley rats, 300 to 350 gm (Canadian Breeding Farms; Quebec, Canada), were individually housed with food always available. Each subject was anesthetized with ether and implanted with an oral cannula consisting of a small-diameter polyethylene tube (Clay-Adams, "intra-medical," P.E. 205) passed under the skin with one end exiting through the skin at the back of the neck and the other entering the oral cavity on the right side just anterior to the molar teeth. The two ends of the cannula were flared and held in place by polyethylene washers and by a thin wire attached to the oral end and looped around subcutaneous tissue in the cheek.

Adaptation. -- Following 3 to 4 days recovery from the cannulation, access to water was limited to 1 hr each day for 3 days. For the next 6 days, water was provided only by infusion into the oral cavity via the cannula, using a Harvard Model 941 pump set to produce a flow rate of 1 ml/min. Daily infusions of 20 ml in four 5-ml segments separated by 2 min permitted adapted subjects to drink the infused water comfortably.

Conditioning. -- One conditioning trial followed the 6-day adaptation period. Ten ml of a 0.2% sodium saccharin solution were infused into the oral cavity at 1 ml/min in two 5-ml segments separated by 2 min. (In order to measure how much of the infused solution was not ingested, subjects were placed in metabolism cages. The slow infusion speed used made it unnecessary to use a restraint as in Experiment 2.) Nineteen subjects received the saccharin via the cannula after having been water deprived for 23 1/2 hr (D); the other 18 subjects received the saccharin after having 20 ml of water infused into the oral cavity as during adaptation (W). Ten subjects in each condition were injected intraperitoneally with 2 ml of 0.12 M lithium chloride (Li) after the saccharin presentation, while the remaining subjects were injected with 2 ml of isotonic sodium chloride (Na). Thus, there were four groups: deprived-lithium (D-Li, $\underline{n}=10$), deprived-sodium (D-Na, $\underline{n}=9$), watered-lithium (W-Li, $\underline{n}=10$), and watered-sodium (W-Na, $\underline{n}=8$).

Ninety min after the conditioning trial, subjects received access to water for 1 hr in the home cage.

Testing. -- Starting the day after conditioning, preference for saccharin versus water was measured in six daily 1-hr preference tests while subjects were 23 hr water deprived. The position of the two solutions was alternated on succeeding days, and preference was measured as the percentage of the total liquid intake consisting of saccharin.

All two-group comparisons were made with the Mann-Whitney U test (two-tailed).

Results and Discussion

Subjects conditioned in a reduced drive state (Groups W-Li and W-Na) drank as much water before conditioning (median = 6.2 ml/100 gm body weight) as comparable subjects in Experiment 2 (Groups WF-Li and WF-Na: median = 6.0 ml/100 gm body weight) $p > .10$). Groups conditioned after 23 1/2 hr of water deprivation (D-Li and D-Na) ingested more saccharin during conditioning than each of the watered groups (median = 10.0 ml range = 9.2 to 10.0 ml; all $ps < .01$). However, despite the reduced water deprivation of Groups W-Li and W-Na, these subjects still ingested a median of 7.0 ml of saccharin (range = 2.5 to 10.0 ml). Thus, with saccharin infused slowly into the oral cavity of freely moving subjects during conditioning, a reduction in deprivation did not suppress ingestive behaviors very much.

The results of the six postconditioning test sessions are shown in Figure 4. Each lithium-injected group (D-Li and W-Li) had lower saccharin preferences than each of the control groups (D-Na and W-Na; all $ps < .02$). The two poisoned groups, however, were not significantly different from each other during any test session (all $ps > .10$), and

Figure 4

Experiment 3: Saccharin preference scores of subjects conditioned either while 23 1/2 hr water deprived (Groups D-Li and D-Na) or after access to 20 ml of water (Groups W-Li and W-Na). Groups D-Li and W-Li received lithium injections during conditioning, while Groups D-Na and W-Na received saline injections.

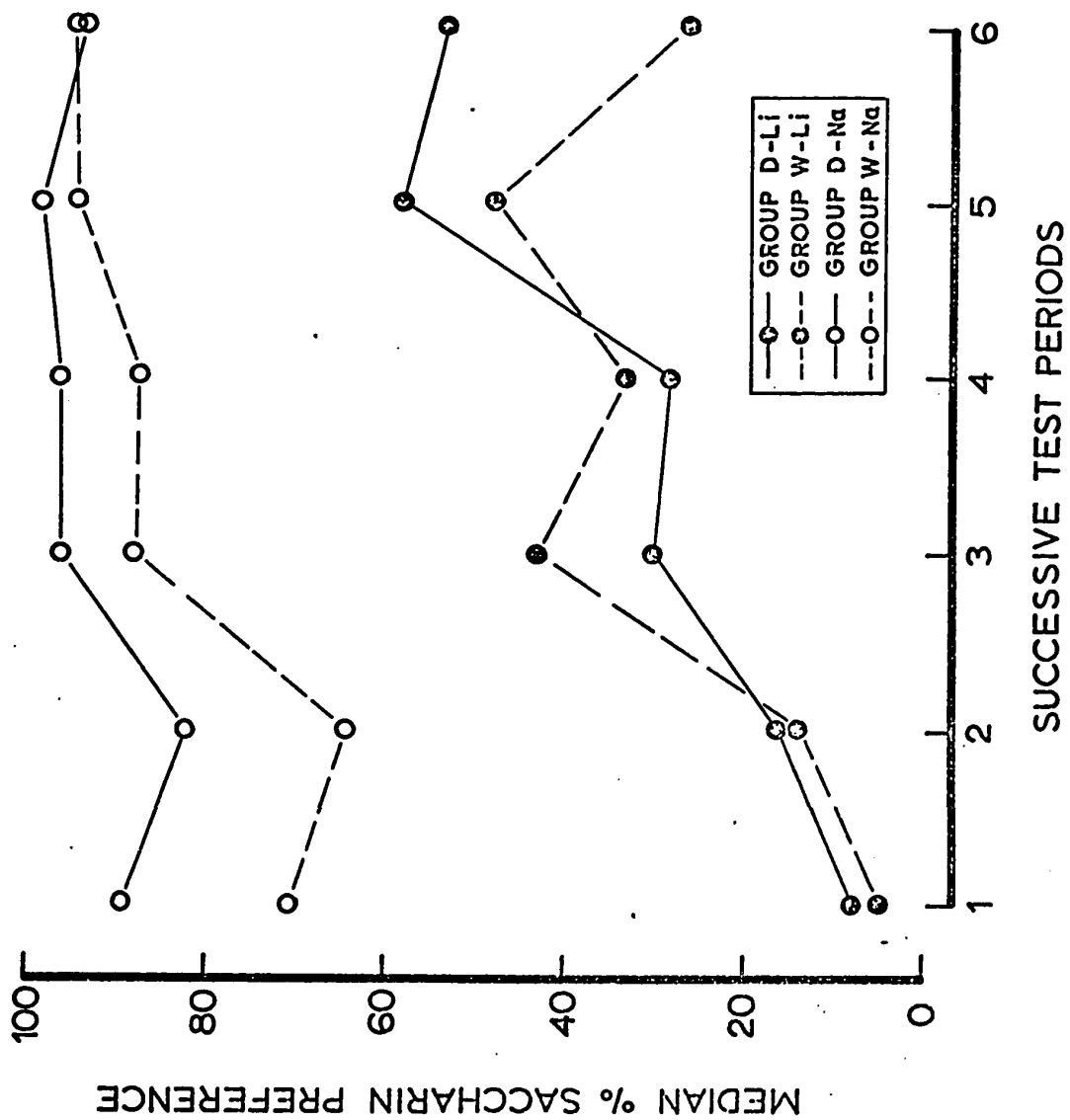


Figure 4

there were no significant differences between the two control groups (all p 's $>.10$). There were also no significant differences in total liquid intake among the groups during the test sessions.

The present results indicate that a reduction in water deprivation unaccompanied by a substantial suppression of ingestion does not attenuate taste-aversion learning. This outcome suggests that the weakened aversion learning of subjects in Group WF-L1 of Experiment 2 was a result of the absence of ingestive behaviors during conditioning rather than a result of reduced thirst.

Experiment 4: Specificity of cue to consequence in aversion learning with CSs presented in the absence of ingestive behaviors I. Compound stimulus conditioning.

Experiments 4 and 5 were designed to explore whether taste stimuli experienced in the absence of approach and ingestive behaviors are less effective in associations with toxicosis because they are perceived as other stimuli which are ordinarily experienced without ingestion, such as audiovisual or spatial cues. Although such tele-receptor cues can become associated with poisoning (e.g. Garcia, et al., 1957), such associations often require higher doses of the toxin unconditioned stimulus (Garcia, et al., 1961). And, the introduction of a 30-min delay between CS and US greatly interferes with the association of nongustatory cues with illness but does not appreciably disrupt taste-aversion learning (Rozin, 1969).

The experimental design followed closely that of Garcia and Koelling (1966) in comparing aversion learning to gustatory and non-gustatory stimuli using toxicosis and foot-shock as unconditioned

stimuli. The design differed from that of the Garcia-Koelling study, however, in that both the gustatory and the nongustatory cues were presented in the absence of approach and ingestive behaviors during conditioning. (Garcia and Koelling had presented both the taste and audiovisual cues consequent to ingestion during conditioning.)

Results of the two previous investigations comparing aversion learning to taste and telereceptor cues paired with toxicosis and foot-shock (Garcia and Koelling, 1966; Garcia, et al., 1968) showed that telereceptor cues are readily associated with shock but not poisoning whereas ingested taste stimuli are readily associated with toxicosis but not shock, a pattern of results that has since come to be referred to as the specificity of cue to consequence effect. If noningested taste stimuli are perceived as telereceptor cues which are readily associated with shock, noningested tastes should also be conditionable to foot shock. Thus, the specificity of cue to consequence effect would not be expected when noningested taste and telereceptor cues are compared in associations with shock and toxicosis.

A saccharin solution served as the taste CS and a buzzer served as the telereceptor CS. Both stimuli were presented independently of the behavior of the subject in the absence of ingestion, the taste CS being administered by the rapid oral infusion technique which was shown to suppress ingestion in Experiment 2. As in the study by Garcia and Koelling (1966), the taste and nongustatory CSs were given equal opportunity for conditioning by being presented simultaneously during training in Experiment 4, and independent groups of subjects had this compound stimulus followed by either shock or poisoning. In order to

increase the possibility for learning taste-shock and buzzer-toxicosis associations -- associations which on the basis of previous work (Garcia and Koelling, 1966; Garcia, et al., 1968) would not be expected to be readily acquired -- three conditioning trials were administered instead of the single one used in Experiments 1, 2, and 3, and the dose of the toxin was increased.

Method

Subjects and Preexperimental Preparation. -- Eighteen male Sprague-Dawley rats (Holtzman Co.; Madison, Wisconsin), 250 to 300 gm housed individually with food always available, were used. Each subject was implanted with an oral fistula similar to that used in Experiment 3. Each subject also had a safety pin implanted in the skin of the back to serve as one of the poles for contact with the shock source.

Starting four days after cannulation, access to water was limited to 35 min/day.

Adaptation. -- Three days of adaptation began after four days on the water deprivation schedule. Each subject had 35 ml of water infused into the oral cavity each day via the cannula at 1 ml/sec followed by 10 ml of water infused at 2 ml/sec. (It should be noted that since these infusion speeds exceed the "Fast" speed used in Experiment 2 (46 ml/min), they would be expected to be even more effective in suppressing ingestive behaviors.) The infusions were carried out after subjects had been allowed their daily access to water for 35 min. Of this 35-min access to water, the first 15 min were provided during the last two days of adaptation by placing

subjects in a test chamber with two drinking tubes filled with water.

Conditioning. -- Each of the three daily conditioning trials was conducted after subjects had been watered for 35 min in the home cage. The auditory CS was provided by an irregularly pulsed buzzer (mean = 4.6 times/sec) which added 20 dB to the 50 dB (SPD) background noise. The gustatory CS was provided by the oral infusion of a 0.2% sodium saccharin solution at 1 ml/sec. The two CSs were presented simultaneously for 35 sec the taste CS being terminated by the oral infusion of 10 ml of water at 2 ml/sec. Immediately after presentation of the CSs, one group ($n=6$) was injected intraperitoneally with 4 ml of 0.12 M lithium chloride, a toxin. Another group ($n=6$) was shocked for 0.5 sec and then injected with 4 ml of isotonic saline (sodium chloride), while the remaining six Ss served as a control group and were only injected with isotonic saline. The shock (140 V, ac) was administered by attaching one pole of the source to the metal conditioning chamber and the other to the safety pin implanted in the dorsal skin of each subject.

Testing. -- Starting the day after the last conditioning trial, two daily 15-min preference tests were conducted, each followed by 20 min of water in the home cage. The test chamber had two drinking tubes. For saccharin preference tests one tube was filled with saccharin and the other with tap water. For buzzer preference tests, both drinking tubes were filled with tap water and the buzzer was activated by licks from one of the two tubes. Subjects were tested once with each CS, the order of these tests counterbalanced in each group. Preference was measured as the percentage of total fluid consumption consisting of the

CS solution. All two-group comparisons were made with the Mann-Whitney U test (two-tailed).

Results

As in Experiment 2, the method of saccharin presentation used exposed subjects to the saccharin flavor in the absence of ingestive responses. Careful observation revealed no drinking movements while the saccharin was rapidly infused into the oral cavity of the non-water-deprived rats. (Specific measurements of the amount of saccharin ingested were not made, since such measurements had been taken under similar conditions in Experiment 2).

Only subjects conditioned with lithium toxicosis learned to avoid the saccharin flavor. Figure 5A shows that the saccharin preferences of lithium-injected subjects were lower than that of both saline-injected and shocked groups ($p < .01$), which did not differ from each other ($p > .40$). In contrast, only subjects conditioned with shock learned to avoid the buzzer. Figure 5B shows that the buzzer-water preferences of shocked subjects were lower than that of both the lithium- and saline-injected groups ($p < .01$), which did not differ from each other ($p > .40$). Thus, as expected from the results of Garcia and Koelling (1966), the saccharin flavor became associated with toxicosis but not shock whereas the buzzer became associated with shock but not toxicosis.

No significant differences in total intake were observed on the buzzer test day. However, lithium-injected subjects drank significantly less fluid on the saccharin test day than saline controls ($p < .05$).

Discussion

The results of the present experiment are consistent with the

Figure 5

Experiment 4: Saccharin and buzzer-water preferences of subjects exposed to both saccharin and the buzzer in conditioning with lithium, shock, or saline.

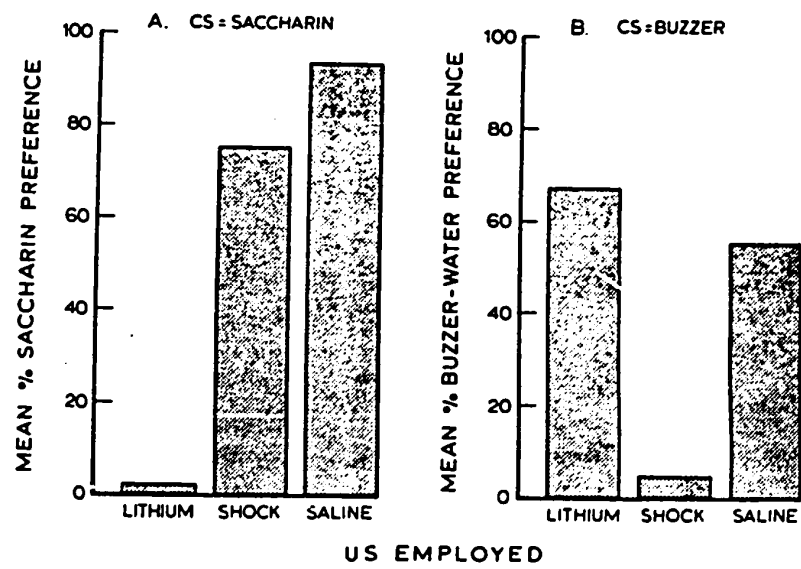


Figure 5

findings of Garcia and Koelling (1966) and further demonstrate that the specificity of cue to consequence effect in aversion learning is independent of the method used to present the two CSs. The taste of saccharin was more readily associated with toxicosis than the sound of the buzzer, and the auditory cue served as a better CS for shock than the taste cue, even though both CSs were presented in the absence of ingestion.

The present results also show that gustatory cues experienced in the absence of ingestive behaviors do not become readily associated with shock. No detectible avoidance of saccharin was observed as a consequence of pairing saccharin with shock during conditioning. The lack of association between the taste of saccharin and shock was not a result of ineffective conditioning parameters, since the sound of the buzzer, which was present simultaneously with the saccharin flavor, readily became associated with shock, and saccharin readily became associated with poisoning.

The present results therefore do not support the suggestion that elimination of ingestive behaviors makes subjects perceive gustatory stimuli as similar to other cues usually experienced without ingestion, such as sounds, which become readily associated with shock. Thus, it is unlikely that tastes experienced in the absence of approach and ingestive behaviors are less effective in associations with toxicosis (Experiment 2) because they are perceived as nongustatory cues that signal peripheral pain as opposed to toxicosis.

Experiment 5: Specificity of cue to consequence in aversion learning with CSs presented in the absence of ingestive behaviors II. Single stimulus conditioning.

It might be argued that the specificity of cue to consequence observed in Experiment 4, and in Garcia and Koelling (1966) was a result of the simultaneous presentation of gustatory and auditory CSs during conditioning. Perhaps the saccharin flavor did not become associated with shock because it was somehow "overshadowed" by the buzzer cue. Similarly, the auditory CS might very well have become associated with toxicosis if the saccharin flavor had not been present. An overshadowing or competing stimulus process provides an attractive explanation since the unequal associability of cues and consequences observed in quail was considerably attenuated when the gustatory and visual CSs were presented individually using independent groups of subjects. (Wilcoxon, Dragoin, & Kral, 1971).

Only one previous report on the specificity of cue to consequence effect in aversion learning in the rat involved the individual presentation of taste and nongustatory CSs during conditioning (Garcia, McGowan, Ervin, & Koelling, 1968). However, in that experiment, presentation of the taste CS was contingent on ingestion, while the nongustatory cue was presented independently of ingestive behaviors. Thus, differences in associability of cues and reinforcers might have resulted from differences in the method of CS presentation.

Experiment 5 was designed as a systematic replication of Experiment 4, with gustatory and auditory cues presented individually during conditioning, using independent groups of subjects.

Method

Experiment 5 was identical to Experiment 4 in all unspecified details. Thirty rats were used, each assigned to one of six independent groups. The buzzer served as the CS for three groups, while the taste of saccharin served as the CS for the remaining three groups. Under each CS condition, one group was injected with lithium chloride during conditioning, another group was shocked and injected with isotonic saline (sodium chloride), while the third group served as a control condition for the first two and only received the isotonic saline injections. Originally five subjects were assigned to each group, however, two subjects died before the end of the experiment, one in the buzzer-lithium condition and the other in the buzzer-shock condition.

As in Experiment 4, each subject was tested with both the buzzer and the saccharin flavor. In order to allow comparison of the test results of Experiments 4 and 5, subjects in Experiment 5 were equated for pre-test contact with the two stimuli by being exposed to the cue absent during conditioning 1.5 to 2.5 hr after each conditioning trial. (Subjects in Experiment 4 had been equated for pre-test contact with the two stimuli by being exposed to both during conditioning.)

Results

The pattern of results obtained was identical to that of Experiment 4. Figure 6A shows that the saccharin flavor readily became associated with lithium toxicosis ($p < .01$ for comparison with saline treatment), whereas shock did not ($p > .50$ for comparison with saline treatment). In contrast, the buzzer readily became associated with shock (see Fig. 6B) ($p < .05$ for comparison with saline treatment),

Figure 6

Experiment 5: (A) Saccharin preferences of subjects exposed to saccharin in conditioning with lithium, shock, or saline.

(B) Buzzer-water preferences of subjects exposed to the buzzer in conditioning with lithium, shock, or saline.

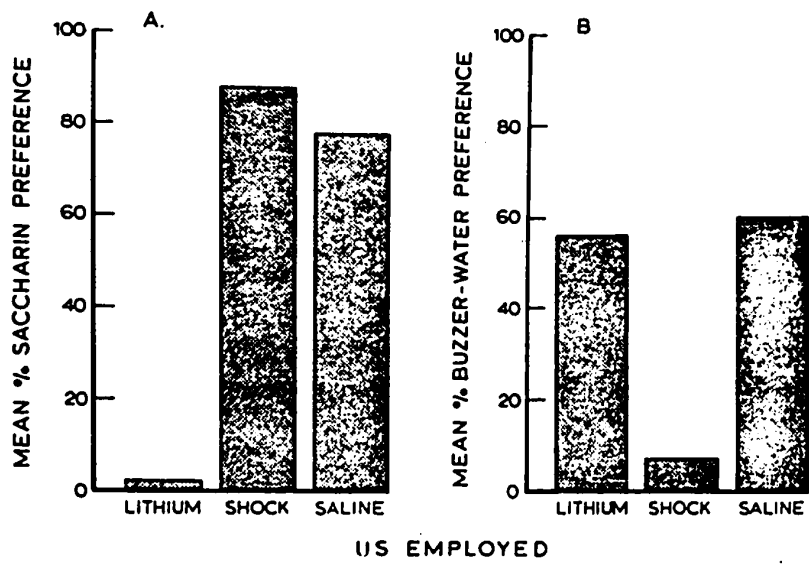


Figure 6

whereas lithium toxicosis did not ($p > .50$ for comparison with saline treatment). (The three groups conditioned with the buzzer did not differ in preference for the saccharin flavor, and the three groups conditioned with saccharin did not differ in preference for the buzzer.)

Discussion

The results of Experiment 5 confirm the specificity of cue to consequence effect observed in Experiment 4. Experiment 5 also demonstrates that this effect is not a result of an "overshadowing" or competing stimulus process. Gustatory cues did not become associated with shock even though no competing novel auditory or visual cues were present, and auditory cues did not become associated with toxicosis despite the absence of novel gustatory cues. The present results therefore confirm that elimination of ingestive behaviors does not make subjects perceive gustatory stimuli as similar to other cues usually experienced without ingestion and provide additional evidence against the hypothesis that tastes experienced in the absence of approach and ingestive behaviors are less effective in associations with toxicosis (Experiment 2) because they are perceived as nongustatory cues that signal peripheral as opposed to visceral pain.

It should be noted that the results of Experiments 4 and 5 do not preclude the possibility that with repeated conditioning trials tastes experienced in the absence of ingestive behaviors may become associated with shock. However, it seems clear that even with the elimination of ingestive behaviors, taste cues are not nearly as associable with foot-shock as auditory stimulation.

Summary and General Discussion

Unlike most forms of exteroceptive stimulation, taste is usually experienced only as a consequence of active ingestive behaviors. Also unlike most other forms of stimulation, taste appears to be uniquely favored in associations with toxicosis in the rat. The experiments reported in this chapter were therefore designed to evaluate the role of ingestive behaviors in the formation of taste-toxicosis associations.

In Experiment 1, taste stimulation was isolated from approach and ingestive responses by presenting a saccharin flavor to rats restrained from skeletal movement by curarization. Curarized subjects experiencing the flavor of saccharin prior to toxicosis subsequently showed greater aversions to saccharin than saline-injected controls or subjects poisoned before presentation of the saccharin flavor. This result demonstrates that ingestive behaviors do not have to accompany gustatory stimulation for the formation of taste-toxicosis associations. The experience of taste alone prior to poisoning is a sufficient condition for taste-aversion learning.

Subjects conditioned while curarized in Experiment 1 formed weaker aversions to saccharin than subjects poisoned after freely ingesting the saccharin solution in the normal state. While this result might suggest that ingestive behaviors facilitate aversion learning, other interpretations are equally plausible since the curarization procedure had a variety of effects in addition to suppressing ingestive behaviors.

In Experiment 2, taste was isolated from ingestive behaviors in noncurarized rats implanted with oral cannulas by reducing the

level of water deprivation and infusing the saccharin very rapidly (46 ml/min) into the oral cavity. Complete suppression of ingestive responses was associated with an attenuation of aversion learning not attributable to either the infusion speed used (see control groups in Experiment 2) or the level of water deprivation used (see Experiment 3). This finding suggests that ingestive behaviors facilitate acquisition of flavortoxicosis associations in the rat.

Only complete elimination of ingestive responses during conditioning resulted in an attenuation of taste-aversion learning. A reduction in the amount of the CS solution ingested during conditioning from 4.5 to 2.0 ml (Experiment 2) or from 10.0 to 7.0 ml (Experiment 3) had no appreciable effect on subsequent aversions. (Similar evidence was obtained by Smith and Morris (1963).) Thus, the facilitory effect of ingestion on taste-aversion learning does not depend on the consumption of large quantities of the CS solution.

Experiments 4 and 5 provided evidence against the hypothesis that tastes in the absence of approach and ingestive behaviors are less effective in associations with toxicosis because they are perceived as other stimuli ordinarily experienced without ingestion which signal peripheral pain rather than visceral upset, such as auditory cues. As expected on the basis of earlier work with ingested taste stimuli (Garcia & Koelling, 1966; Garcia, et al., 1968), gustatory cues experienced in the absence of ingestive behaviors failed to become associated with foot-shock, whereas auditory cues paired with shock readily acquired aversive properties.

The taste-aversion learning of subjects conditioned in the absence of approach and ingestive behaviors may have been attenuated because rats have not evolved effective mechanisms to learn about flavors they do not approach and ingest. This is not an unlikely possibility since novel flavors are hardly every experienced in the absence of approach and ingestion in the natural history of the rat. However, this hypothesis does not easily lend itself to experimental verification.

Another possible explanation for the attenuated taste-aversion learning of subjects conditioned in the absence of ingestive behaviors is that in their developmental history the subjects had not learned to be alerted to possible visceral consequences after experiencing non-ingested novel flavors, whereas they had had the opportunity to learn that ingesting novel flavored substances could lead to significant postingestional effects. This explanation is similar to the evolutionary hypothesis suggested above except that it attributes the weakened taste-aversion learning of no-ingestion subjects to individual rather than evolutionary history. Investigation of this hypothesis would require that rats be raised and nourished experiencing thirst and hunger reduction as well as other postingestional effects in conjunction with flavored substances that are tasted but not ingested.

Although the weakened taste-aversion learning observed when subjects were conditioned in the absence of ingestive behaviors may have reflected individual or evolutionary history, a third possibility is that the effect resulted from a generalization decrement process. Perhaps substances that are tasted but not ingested have a flavor

slightly different from the flavor of substances actively consumed. All groups were tested drinking the flavored CS solution. Thus, for subjects conditioned in the absence of ingestion the flavor experienced during conditioning may have been different from the flavor experienced during the test sessions. If this generalization decrement hypothesis is correct, subjects conditioned in the absence of ingestion should not show attenuated taste aversions if the CS flavor is also experienced in the absence of ingestive behaviors during the postconditioning tests. However, attenuated taste aversions would be expected in no-ingestion tests for subjects poisoned after drinking the CS solution.

Experimental investigation of the generalization decrement explanation of the attenuated taste aversions observed in no-ingestion subjects therefore requires the development of a no-ingestion test of taste aversions. Such a task is potentially beset by many difficulties. For example, rats that are less able to associate noningested tastes with toxicosis may also be less able to associate the termination or onset of a noningested taste with some nonconsumatory response such as locomotion or lever pressing. Their inexperience with noningested flavors may also make noningestion tests of taste aversion learning far less sensitive than ingestion tests.

Potential difficulties in studying the role of ingestion in poison-avoidance learning encountered because of the unusual nature of taste stimuli presented in the absence of ingestive behaviors, could possibly be minimized by using a poison-avoidance learning preparation which does not involve taste as the conditioned stimulus. For example, visual cues are effective as conditioned stimuli in the poison-avoidance

learning of birds (e.g., Brower, 1969; Wilcoxon, Dragoin, & Kral, 1971). Visual cues can easily be presented both in conjunction with ingestive behaviors as well as in the absence of ingestion simply by controlling access to consumables. And, most importantly, birds encounter visual cues both with and without ingestion in their natural history. A poison avoidance preparation in the rat similar to the visual aversion learning of birds involves the association of odors with poisoning. Odor-toxicosis learning in rats has been reported by Garcia and Koelling (1967) and Lorden, Kenfield, and Braun (1970). Rats encounter odors both in conjunction with, as well as in the absence of, ingestive behaviors in their natural history. And, just as with visual cues, ingestion during the presentation of olfactory cues can be easily controlled by the availability of consumables. Therefore, rather than pursue the problem of the role of ingestion in poison avoidance learning in the rat using the taste-aversion preparation, subsequent experiments (reported in Chapter 3) were designed to explore the role of ingestion in odor-toxicosis learning. This investigation promised not only to further our understanding of the role of ingestion in poison-avoidance learning but also to possibly replicate effects observed in the taste system, thus demonstrating their generality.

CHAPTER 3: ROLE OF INGESTION IN ODOR-TOXICOSIS LEARNING IN THE RAT

In contrast to the considerable experimental literature on taste-aversion learning (see reviews by Garcia & Ervin, 1968; Revusky & Garcia, (1970), there has been little research on learning odor-toxicosis associations. In several early experiments on learned odor aversions, odor cues were presented by dissolving the odorizing agent in a solution ingested by the subjects during both conditioning and testing (e.g., Lovett, Goodchild, & Booth, 1968; Pain & Booth, 1968). These studies do not provide conclusive evidence of odor-toxicosis learning since dissolving the odorizing agent in an ingested solution may allow subjects to respond to the solution on the basis of taste rather than smell. More convincing evidence for odor-aversion learning is provided by studies in which the odorizing agent was located near a drinking tube rather than dissolved in the solution ingested (Garcia & Koelling, 1967; Hankins, Garcia, & Rusiniak, personal communication; Lorden, Kenfield, & Braun, 1970).

It is interesting to note that in all experiments purporting to demonstrate odor-aversion learning, subjects were permitted to drink fluids while experiencing the CS odor prior to toxicosis. Postconditioning test procedures typically also involved the drinking response. Odor aversions were assessed in terms of either suppression of drinking in the presence of the CS odor (e.g., Garcia & Koelling, 1967) or suppression of a lever-press response which had been previously reinforced with access to fluids (Lorden, et al., 1970).

The choice of conditioning and test procedures involving ingestive behaviors to study odor-toxicosis learning may not have been

entirely arbitrary. Perhaps rats learn aversions to olfactory cues only if they experience the odors in conjunction with ingestion during conditioning. And, perhaps rats show aversions to odors associated with toxicosis only in tests involving ingestion. That is, odors experienced in the context of ingestion-associated stimuli may more likely be treated as relevant to visceral consequences than odors experienced without ingestion.

Garcia and Ervin (1968) have pointed out that olfactory stimulation sometimes acts within the gustatory-visceral system providing chemical information about food while at other times it is a part of the tele-receptor-cutaneous system, providing information about, for example, predators. Olfactory stimulation per se is therefore ambiguous to the rat. Additional information is required before an arbitrary odor can be interpreted as relevant to either visceral or cutaneous events. The presence or absence of stimuli associated with ingestion may provide some of this additional information. The experiments reported in Chapter 3 were designed therefore to evaluate the role of ingestion in odor-toxicosis learning.

Experiment 6: Odor-aversion learning with and without ingestion of water during conditioning.

The purpose of Experiment 6 was to see if odors experienced in conjunction with ingestion-associated stimuli are more readily associated with toxicosis than odors experienced in the absence of ingestion. The design of Experiment 6 was similar to the design of Experiments 1 and 2, which were conducted to answer the same question in taste-aversion learning. Ingestion during exposure to the CS odor was manipulated by

controlling access to consumables, in this case water. The aversion learning of subjects drinking water during exposure to the CS odor prior to toxicosis was compared to the aversion learning of subjects experiencing the odor in the absence of ingestion. To insure the same exposure to the odor in ingestion and no-ingestion conditions, subjects were placed in chambers permeated with a scent. Drinking therefore did not take subjects closer to the odor source, as had been the case in most previous studies of odor-aversion learning.

Method

Subjects and Apparatus. -- Thirty-two male albino Charles River rats, 200-250 gm, were individually housed with continual access to Purina Laboratory Chow and 30-min daily access to water. Plastic pails with lids, a wire mesh floor over absorbant bedding, and a hole for inserting a drinking spout about 5 cm above the floor served as experimental chambers. The pails used during adaptation were 34.3-cm tall, with a volume of approximately 18700 cm³ and had loosely fitting lids. Smaller but similar pails with tightly fitting lids were used as conditioning and testing chambers (height=39.0 cm, volume=15500 cm³). The CS odor was provided by the scent of "Mentholatum" cream (Mentholatum Co., Fort Erie N., Ontario, Canada) spread generously on the inside surface of the conditioning and testing chamber lids. (The odor of Mentholatum has previously been found to be a salient stimulus in learning experiments (Woods, Markous, & Hutton, 1969).)

Procedure. -- After at least three days on the maintenance schedule, subjects received four adaptation sessions, conducted on succeeding days, during which they were allowed to drink water in the

adaptation chambers for 15 min, followed by 15 min of water in the home cage.

The day after the last adaptation session, subjects were placed in the conditioning chambers permeated with the scent of Mentholatum. After 7 min in the chambers, each subject received a 2% body weight intraperitoneal injection of either 0.12 M lithium chloride, a toxin, or isotonic sodium chloride. Sixteen subjects had water available in the conditioning chambers (Ingestion), while the other 16 experienced the odor of Mentholatum in the absence of consumables (No-Ingestion) during conditioning. Eight of the Ingestion and No-Ingestion subjects were conditioned with the lithium toxin while the remaining eight were conditioned with the control sodium chloride injections. No-Ingestion subjects were permitted to drink water in the home cage just before being placed in the conditioning chambers so that they would not be more water deprived than Ingestion subjects when receiving the conditioning injections. The amount of water No-Ingestion subjects were allowed was determined by yoking each No-Ingestion subject to an Ingestion subject receiving the same conditioning injection. This yoking procedure necessitated that each Ingestion subject be run before its No-Ingestion partner. Immediately after the conditioning injections, subjects were returned to their home cages.

All subjects received their daily 30 min of water 90 min after conditioning. The next day, after 23-24 hr of water deprivation, subjects were tested for aversion to the Mentholatum odor by being allowed to drink water in the odorized testing chambers for 15 min, with intakes recorded every 30 sec (\pm .2 ml). Two days later subjects received a

second conditioning trial conducted as the first. Odor aversions were then assessed in 15-min water drinking tests conducted on successive days. Each test session was followed by 30 min of water in the home cage.

Two conditioning and testing chambers were used for each group. The bedding was replaced after each subject to minimize transfer of the odors of one subject to the next, and each chamber was used no more than once every 30 min, allowing at least 15 min between subjects during both conditioning and testing. After being run in one of the odorized chambers each subject was returned to its home cage placed about 4 m away from subjects that had not yet been tested that day.

All two-group comparisons were made with the Mann-Whitney U test (two-tailed).

Results

Ingestion subjects drank a mean of 10.2 ml while in the odorized chambers for 7 min prior to the first conditioning injections. Fourteen of the 16 No-Ingestion subjects took less than 7 min (median=4.4 min) to drink the same amount of water in the home cage just before being placed in the conditioning chambers ($p < .01$). Since subjects received extensive adaptation to drinking in pails similar to the conditioning chambers, the shorter drinking times of No-Ingestion subjects suggest that the Mentholatum odor had a slight unconditioned suppressing effect on water intake.

Intakes during the 15-min drinking test after the first conditioning trial are presented in Figure 7. Non-poisoned subjects drank considerably more than poisoned subjects ($p < .01$ at 15 min), but Ingestion and No-Ingestion groups did not differ from each other whether they were

Figure 7

Experiment 6: Cumulative water intakes of Ingestion and No-Ingestion poisoned (Li) and control (Na) subjects in the presence of the odor of Mentholatum after the first conditioning trial.

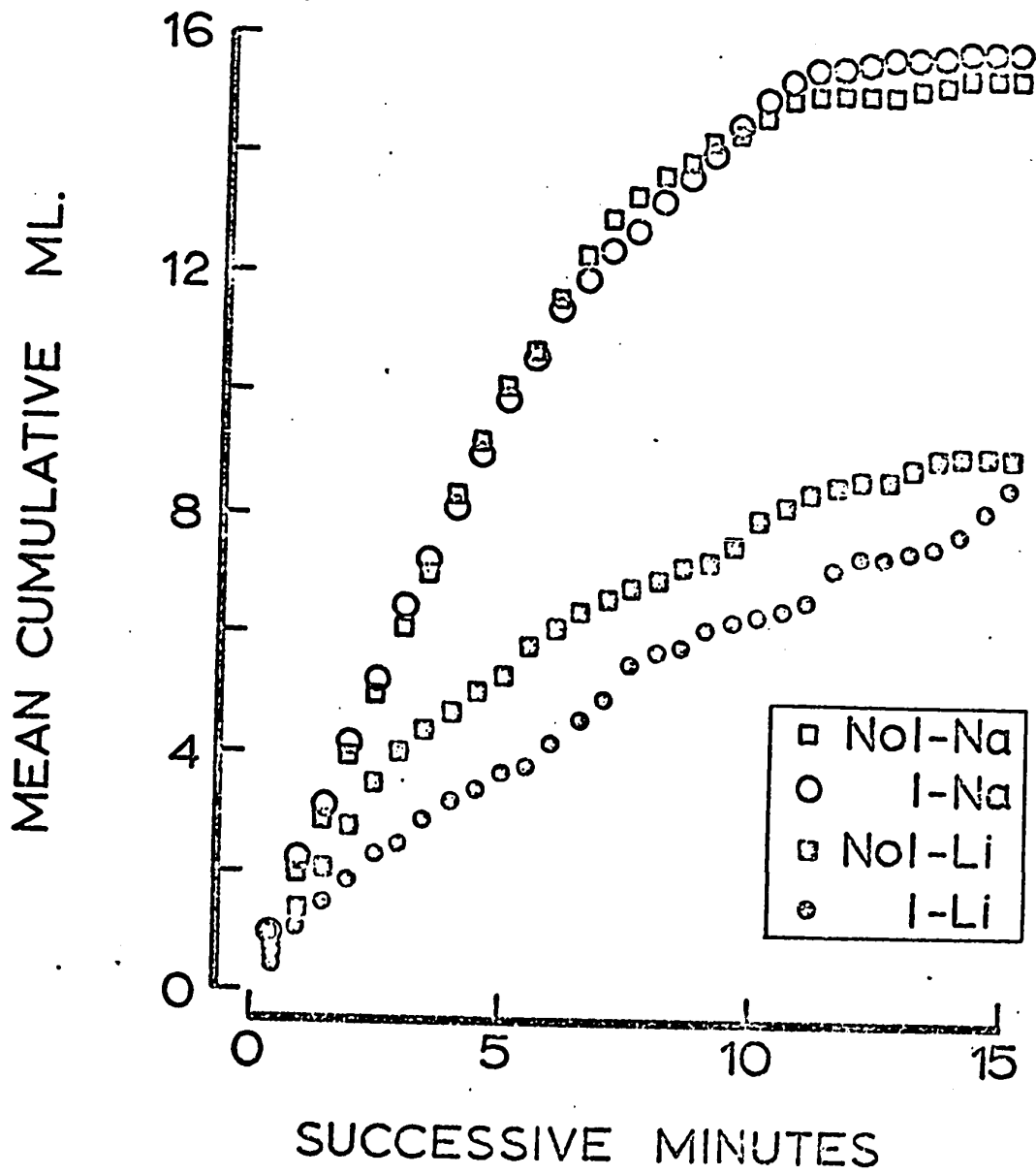


Figure 7

poisoned or not.

Results of the two test sessions conducted after the second conditioning trial are presented in Figure 8. Again, both poisoned groups drank less than non-poisoned controls ($p < .01$ at the end of Test 1 and $p < .02$ at the end of Test 2), and non-poisoned Ingestion and No-Ingestion subjects did not differ from each other. In contrast with first conditioning trial, however, poisoned Ingestion subjects drank less water in the Mentholatum odor than poisoned No-Ingestion subjects throughout each test session. (The difference between the two poisoned groups was significant at the .01 level at the end of Test 1 and at the .03 level at the end of Test 2.)

Discussion

The present results concerning odor-toxicosis learning appear to replicate the findings of Experiments 1 and 2 in taste-aversion learning in showing that ingestion is not necessary but appears to facilitate poison avoidance acquisition. Drinking water during conditioning was not found to be necessary for odor aversion learning. However, Ingestion subjects displayed stronger aversions than No-Ingestion subjects.

Although the suppressed water intakes of poisoned subjects in the present experiment probably reflected learned aversions to the CS odor, acquired aversions to various non-olfactory features of the conditioning chambers, such as visual or tactile cues or the taste of water, also could have resulted in suppression of water intake during the test sessions. While such aversions are not readily learned (e.g., Garcia & Koelling, 1967), conditioned aversions to both environmental cues (Rozin, 1969) and water (Nachman, 1970) have been observed.

Figure 8

Experiment 6: Cumulative water intakes of Ingestion and No-Ingestion poisoned (Li) and control (Na) subjects in the presence of the odor of Mentholatum after the second conditioning trial.

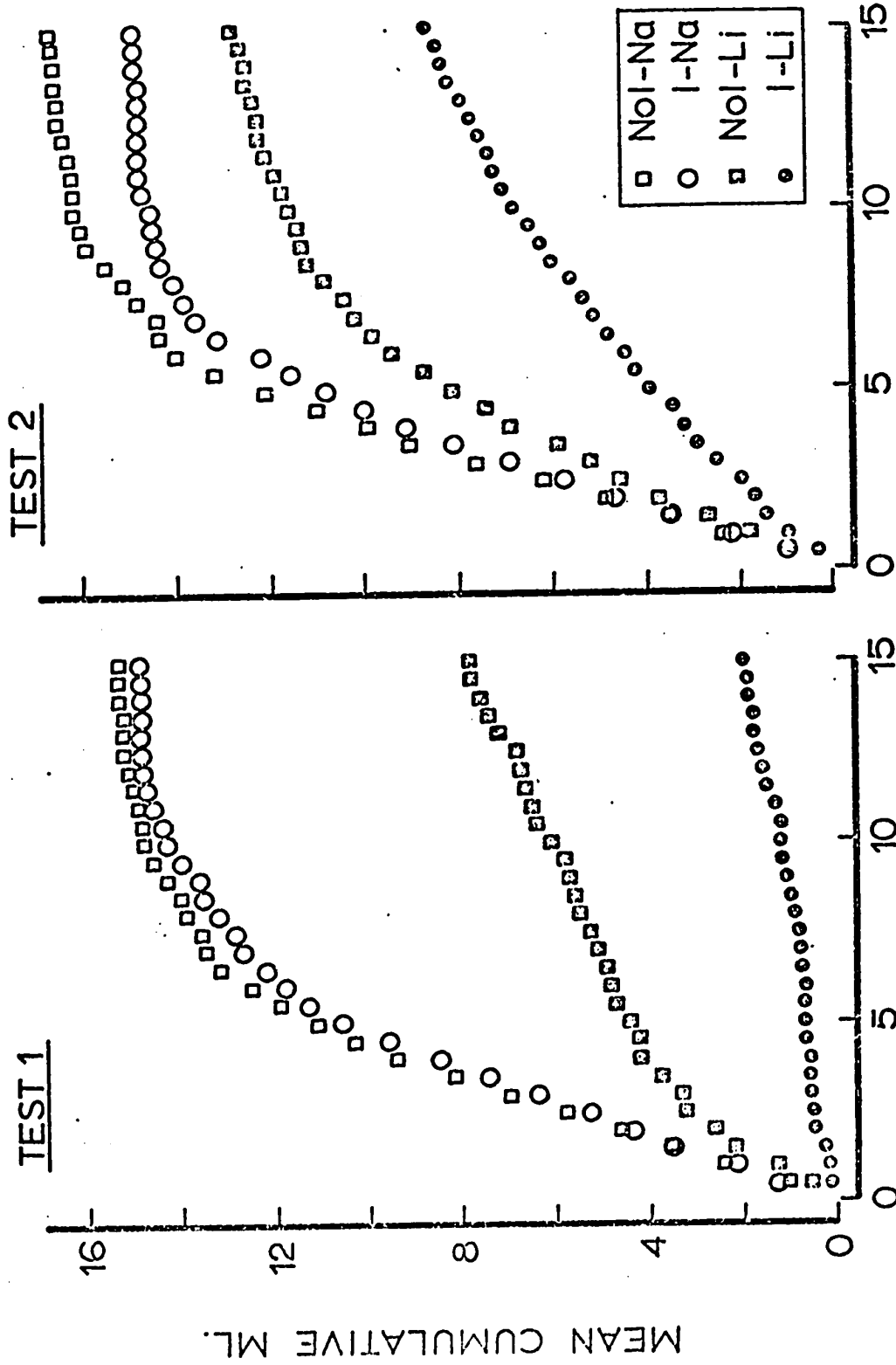


Figure 8

Experiment 7: Odor aversion control experiment I.
Ingestion and No-Ingestion subjects
tested with the CS odor but conditioned
without it.

Ingestion subjects in Experiment 6 had access to water for seven minutes in the conditioning chambers immediately before being injected with the toxin. In contrast, No-Ingestion subjects drank an equal amount of water with a seven-minute delay between their last contact with water and subsequent poisoning. The lack of delay between water intake and toxicosis for Ingestion subjects may have allowed them to learn stronger aversions to water, and this stronger water aversion may have been responsible for the greater suppression of water intake observed in Ingestion poisoned subjects during the postconditioning test sessions. Experiment 7 was designed to assess the contribution of learned aversions to the taste of water to the differential effects of ingestion on aversion learning observed in Experiment 6. Ingestion and No-Ingestion groups were conditioned with toxicosis using procedures identical to those of Experiment 6 with the exception that the Mentholatum odor was absent during conditioning.

Method

The procedure was identical to that of Experiment 6 in all unspecified details. Two groups, each consisting of 8 subjects, were conditioned as Ingestion and No-Ingestion poisoned subjects in Experiment 6 except that conditioning was carried out in absence of the odor of Mentholatum with the adaptation instead of the Mentholated chambers used for training. Since the Mentholatum odor was absent during conditioning, subjects in the Ingestion treatment did not experience the slight unconditioned suppressing effects on water intake of the Mentholatum odor.

Had they been permitted to remain in the training chambers for 7 min as subjects in Experiment 6, they would have drunk more water. The Ingestion group was therefore allowed to remain in the training chambers only as long as it took them to drink as much water as poisoned Ingestion subjects in Experiment 6, to which they had been yoked. (Six of these eight subjects drank the specified amount of water in less than 7 min during both conditioning trials (median=5.0 min for conditioning trial 1 and 6.3 for conditioning trial 2).) As in Experiment 6, No-Ingestion subjects were detained in the training chambers for 7 min just after having drunk as much water in the home cage as their yoked Ingestion partners. The toxic lithium chloride injections were administered when subjects were removed from the training chambers with Ingestion subjects injected immediately after drinking water and No-ingestion subjects injected 7 min later, as in Experiment 6. As in previous experiments all two-group comparisons were made with the Mann-Whitney U-test.

Results and Discussion

Only data from test sessions conducted after the second conditioning trial are discussed since it was only after the second conditioning trial that marked aversions differentially affected by ingestion during conditioning were observed in Experiment 6. Figure 9 shows that Ingestion and No-Ingestion groups did not differ in water intake in the presence of Mentholatum during either the first or second test session. Conditioning subjects in the absence of Mentholatum did not result in differential aversions as a function of ingestion during training. This outcome indicates that the differences in the conditioning

Figure 9

Experiment 7: Cumulative water intakes in the presence of the odor of Mentholatum after the second conditioning trial for Ingestion and No-Ingestion poisoned subjects conditioned without the Mentholatum odor.

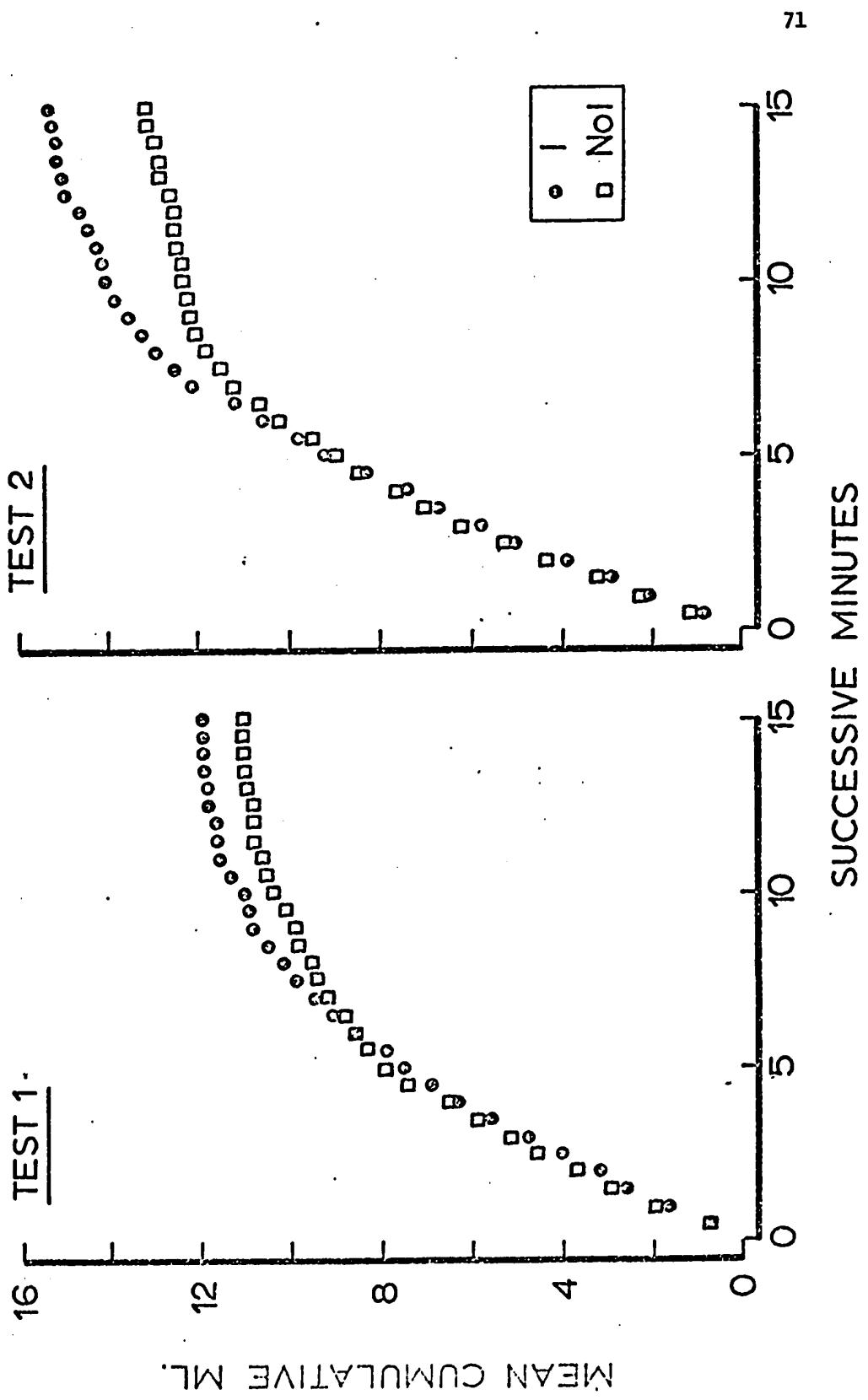


Figure 9

procedures used with Ingestion and No-Ingestion subjects do not result in differences in aversion to water that are evidenced when subjects drink water in the presence of the odor of Mentholatum.

Experiment 8: Odor-aversion control experiment II.
Ingestion and No-Ingestion subjects conditioned with the CS odor but tested without it.

Experiment 8 was designed to further explore the contribution of learned aversions to non-olfactory cues to the differential effects of ingestion on aversion learning observed in Experiment 6. Ingestion and No-Ingestion subjects were conditioned with toxicosis in the presence of the Mentholatum odor using the procedures of Experiment 6. Instead of being tested with the CS odor, however, subjects were given water-intake tests in the absence of the CS odor in chambers identical to those used during conditioning. If the enhanced aversions of Ingestion subjects observed in Experiment 6 reflected aversions to non-olfactory cues, such enhanced aversions also should be evident in tests without the CS odor.

In addition to providing evidence of the contribution of learned aversions to non-olfactory cues, the present experiment was also designed to provide evidence of the specificity of the odor-aversions presumably learned in Experiment 6. Since it is very difficult, if not impossible, to create an odor-free environment, the water-intake tests conducted without the CS odor in the present experiment would be no doubt conducted in the presence of some other unspecified odor. The results of these tests therefore would indicate the extent to which aversions to the CS odor generalize to other olfactory cues.

The procedure was identical to that of Experiment 6 in all unspecified details. Two groups of rats, each consisting of 9 subjects, were conditioned in the presence of the odor of Mentholatum as poisoned Ingestion and No-Ingestion subjects of Experiment 6 but were tested without this CS odor in chambers otherwise identical to those used during conditioning. The no-Mentholatum tests were conducted in a room far removed from the room with the Mentholatum test chambers, and the experimenter did not handle the Mentholatum cream the day that no-Mentholatum tests were to be conducted. To further minimize possible odor contamination during the no-Mentholatum tests, the no-Mentholatum test chambers were ventilated for at least 15 min by a powerful fan before each subject was tested. After conducting the no-Mentholatum tests, subjects were tested with the CS odor to confirm that they had, in fact, learned aversions to the odor of Mentholatum. As in previous experiments, all two-group comparisons were made with the Mann-Whitney U-Tests.

Results

Only results from test sessions conducted after the second conditioning trial are discussed since it was only after the second conditioning trial that marked aversions differentially affected by ingestion during conditioning were observed in Experiment 6. The data are presented in Figure 10.

During Test 1, No-Ingestion subjects drank significantly more water than Ingestion subjects during the first five min ($p < .01$). However, because of increased variability in intake during the latter part of the test session, this difference was not reliable by the

Figure 10

Experiment 8: Cumulative water intakes in the presence and absence of the odor of Mentholatum for Ingestion and No-Ingestion poisoned subjects conditioned with the Mentholatum odor.

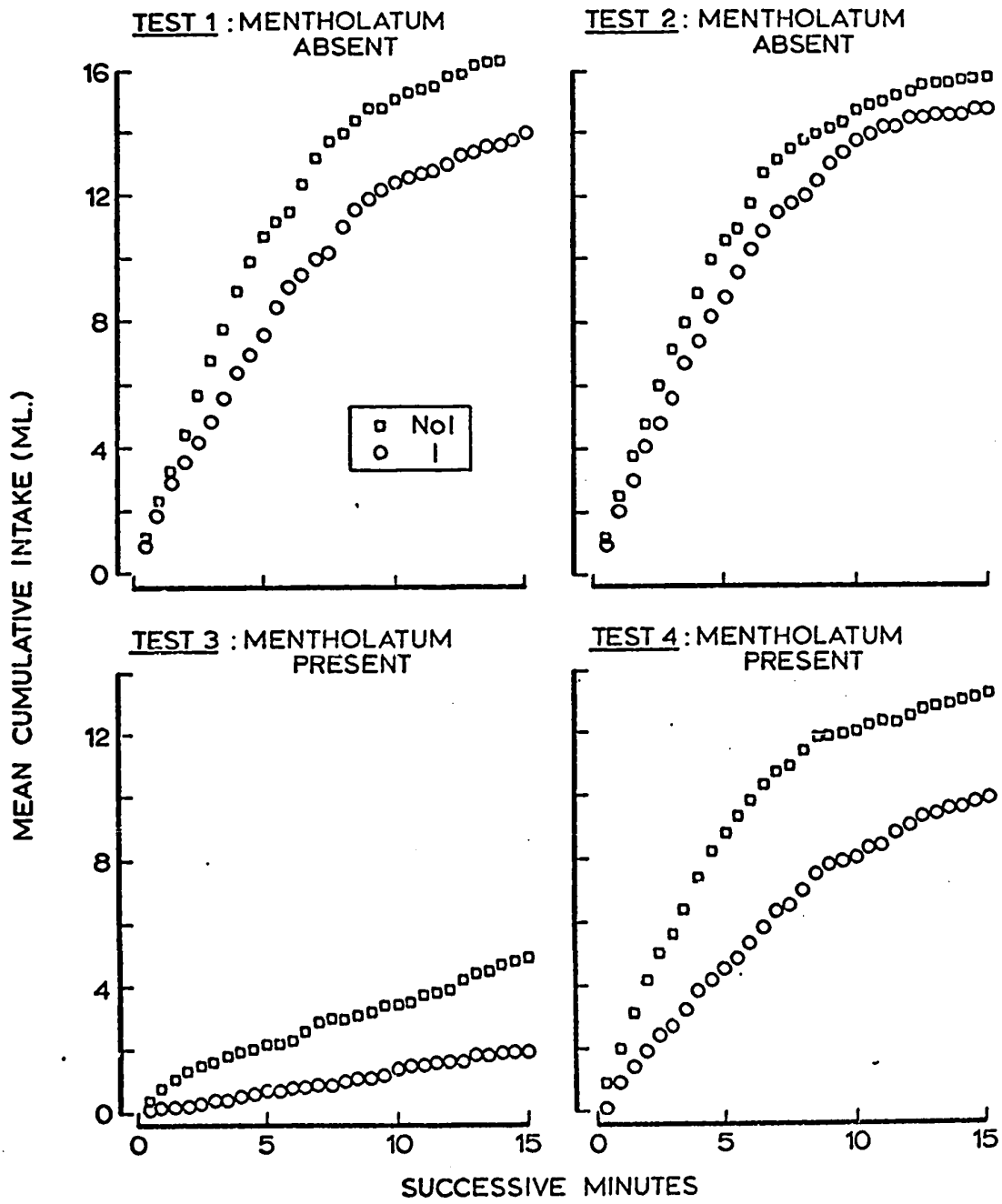


Figure 10

10th min ($p > .05$). Furthermore, the two groups drank comparable amounts throughout the second test without the Mentholatum odor, indicating that the difference which had been observed between them early in the first test session was indeed quite transitory.

These subjects were tested in the presence of the Mentholatum odor the following two days to see whether, in fact, they had acquired differential aversions to the Mentholatum scent as comparable groups in Experiment 6. The water intakes of both groups were considerably suppressed by the Mentholatum odor (see Figure 10, Test 3). (Every subject drank less during Test 3 than Test 2.) In addition, subjects that had been conditioned drinking water in the presence of Mentholatum evidenced significantly more suppression of water intake than No-Ingestion subjects ($p < .05$ at the end of Test 3). Ingestion subjects continued to drink less water than No-Ingestion subjects during the first five minutes of a second test with the Mentholatum odor (see Figure 10, Test 4; $p < .01$), but, as both groups drank increasing amounts during the session, the difference between them failed to reach conventional levels of significance at minutes 10 and 15 because of increased variability in intake among subjects.

Discussion

Ingestion and No-Ingestion subjects conditioned with the Mentholatum odor showed only a transitory difference in water intake when tested in the absence of Mentholatum. This small difference was probably due to the fact that the Mentholatum odor was not the only olfactory cue present prior to toxicosis (the bedding and the plastic of the conditioning chambers were no doubt additional sources of odor),

whereas it was the only cue removed for the test sessions. Although the intakes of Ingestion and No-Ingestion subjects were comparable during the second test in the absence of the Mentholatum odor, Ingestion subjects drank less than No-Ingestion subjects when subsequently tested in the Mentholatum test chambers, and both groups drank less than in immediately preceding tests without Mentholatum. These results provide further evidence that the differential suppression of water intake observed in subjects conditioned and tested with Mentholatum (Experiment 6) was not a result of differential aversions to either water or other non-olfactory features of the conditioning chambers. These findings also show that the aversions learned are quite specific to the presence of Mentholatum. (Additional evidence that the aversions learned in the present experiments were in fact aversions to the odor of Mentholatum is presented in Experiments 12 and 13.)

The facilitory effect of Ingestion on odor-toxicosis learning observed in the present experiment and Experiment 6 is similar to the facilitory effect of ingestion on taste-aversion learning observed in Experiments 1 and 2. Since rats experience odors both in conjunction with ingestion as well as in the absence of ingestive behaviors in their natural history, it is unlikely the weaker odor-aversion learning shown by No-Ingestion subjects was a result of the novelty of experiencing odors in the absence of ingestion. However, the generalization decrement explanation suggested at the end of Chapter 2 to account for the deficit in taste-aversion learning seen in No-Ingestion subjects might also account for the deficit in odor-aversion learning observed in No-Ingestion subjects in the present experiments. Just as in the taste-aversion

experiments, odor aversions in the present experiments were assessed in tests involving ingestive behaviors. Thus, the test situation resembled the training condition of Ingestion subjects more than that of No-Ingestion subjects, and the less marked aversions observed in No-Ingestion subjects may have reflected a generalization decrement from the conditioning to the test situation.

As pointed out at the end of Chapter 2, an adequate test of generalization decrement requires that aversions be assessed both in tests involving ingestive behaviors as well as in tests not involving ingestion. If the generalization decrement hypothesis is correct the relative aversions observed in Ingestion and No-Ingestion subjects should be a function of the nature of the test. Ingestion subjects would be expected to evidence weaker aversions in tests not involving ingestion and No-Ingestion subjects would be expected to show weaker aversions in ingestion tests.

Experiment 9: Comparison of Ingestion and No-Ingestion tests of odor aversions I. Water available during the Ingestion test.

Experiment 9 was designed to assess the extent to which the weaker odor aversions observed in No-Ingestion subjects in Experiments 6 and 8 may have reflected a generalization decrement from the conditioning to the test situation. The odor aversions of Ingestion and No-Ingestion subjects were compared in tests not involving ingestive behaviors as well as in tests involving ingestion.

After conditioning, subjects were given a choice between two compartments, one odorized with Mentholatum and the other left untreated. In one test condition no fluids were available in either compartment

of the odor-choice apparatus, while in another water was available in both compartments. The no-water test condition thus resembled the training procedure of No-Ingestion subjects, whereas the water test condition resembled the training procedure of Ingestion subjects. If subjects evidence stronger aversions in test situations more closely resembling their training condition, No-Ingestion subjects should show greater aversions for the CS odor in the no-water test, while Ingestion subjects should show greater aversions for the CS odor in the water test.

Method

Apparatus. -- The chambers used for adaptation and conditioning were identical to those used in Experiment 6. Aversions to the odor of Mentholatum were tested in an odor-choice chamber, the top view of which is shown in Figure 11. Subjects were placed in area A, and access to compartments B and C was controlled by guillotine doors. Compartments B and C were each provided with an exhaust fan on the left which set up air currents preventing odors in one compartment from reaching the other. (The air was exhausted to the outside of the building.) For odor-choice tests Mentholatum cream was spread on wall D of compartment B. Drinking spouts could be introduced at points F and G about 3 cm above the floor. All walls, made of black Plexiglas, were 18.6 cm high and the top was made of clear plastic.

Subjects and Procedure. -- Twenty-six male albino Charles River rats, 200-250 gm (Quebec Breeding Farms; Quebec, Canada), were individually housed with continual access to Purina Laboratory Chow and 30-min daily access to tap water. Adaptation and conditioning procedures were identical to those of Experiment 6, with subjects given a 15-min

Figure 11

Experiment 9: Top view of odor-choice apparatus.

(Arrows indicate exhaust fans; see text for further details.)

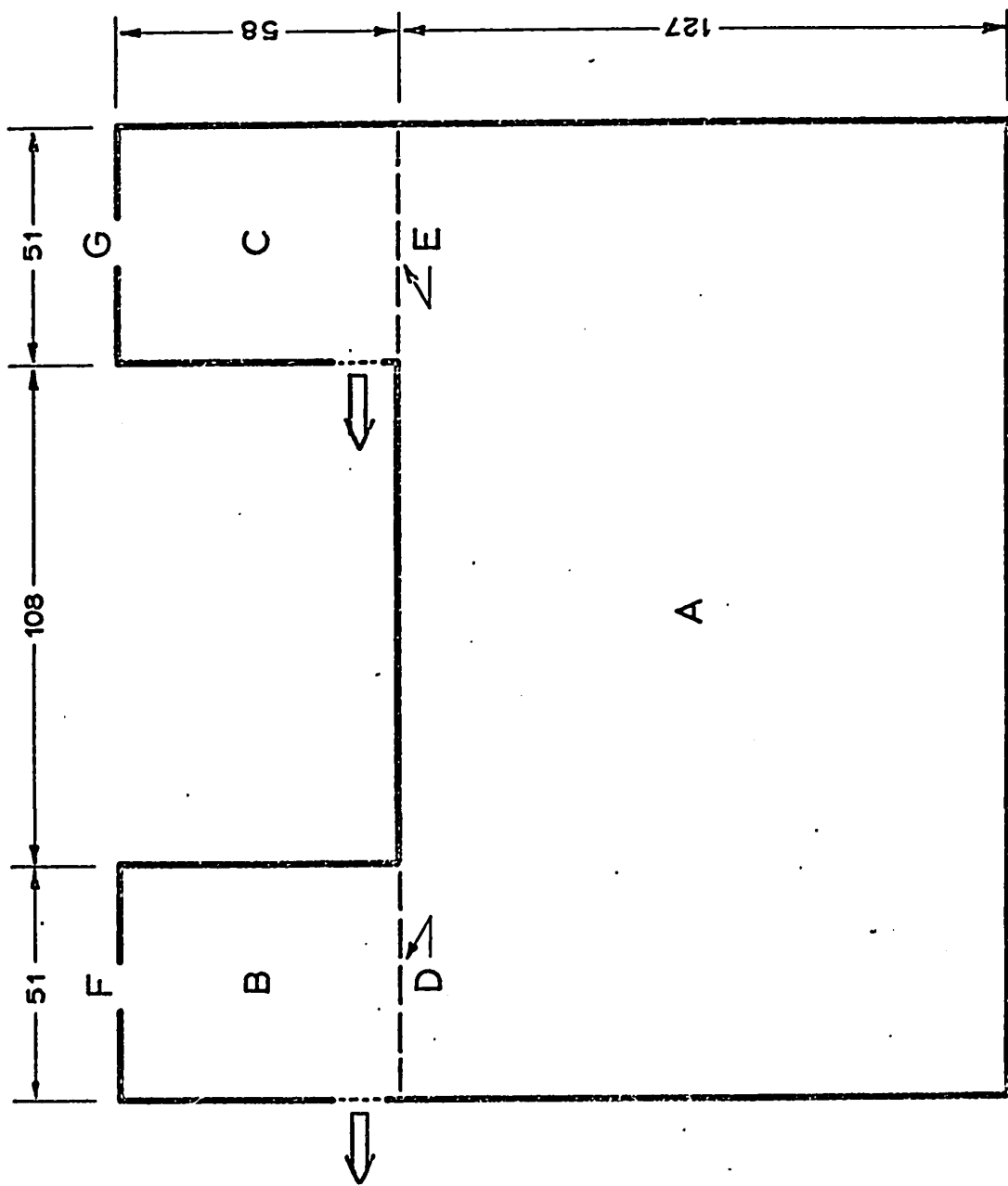


Figure 11

water-drinking test in the conditioning chambers between the first and second conditioning trials. In addition, subjects were allowed to explore the odor-choice apparatus for 3 min the day before the second conditioning trial. (Both compartments were blocked with the guillotine doors during this brief adaptation session.)

All subjects were conditioned after exposure to the Mentholatum odor, 18 with lithium chloride and the remaining 8 with control isotonic sodium chloride injections. Half the subjects receiving each training injection was run in the Ingestion condition. The others, yoked to Ingestion subjects for amount of water drunk prior to the training injections (see Experiment 6), were run in the No-Ingestion condition.

Three test sessions were conducted in the odor-choice chamber on successive days starting the day after the second conditioning trial, with subjects 23-24 hr water deprived before each test. Fresh Mentholatum was used on each test day.

During each test session, subjects were first forced to sample each compartment of the odor-choice chamber, starting with the Mentholatum compartment. The Mentholatum compartment was then opened again, and when the subject entered it, the other side was also opened, both remaining simultaneously available for the next 10 min.

During Test 1 subjects did not have fluids available in either compartment of the test chamber, and the amount of time spent exploring each was recorded by an observer. Tests 2 and 3 were conducted as Test 1 except that water was available in each compartment of the test chamber and intakes were recorded at 1-min intervals.

The experiment was run in one replication with all 26 subjects

given a test session on the same day. After each subject, the test chamber was wiped clean with rubbing alcohol, and fresh paper towel was placed under the chamber to catch any droppings.

All two-group comparisons were made with the Mann-Whitney U test (two-tailed).

Results

A preference score was calculated for each postconditioning odor preference test. For Test 1, when no fluids were available in the test chamber, the preference score was computed by dividing the amount of time spent exploring the Mentholatum compartment by the total time spent exploring both compartments. For Tests 2 and 3, when fluids were available in both compartments, the preference score was computed by dividing the amount ingested in the Mentholatum compartment by the total amount ingested.

The preference scores for each test are shown in Figure 12, with Ingestion and No-Ingestion control subjects presented as one group since they had comparable preference scores in all three tests. Each of the poisoned groups had lower preferences for the Mentholatum compartment during Test 1 than saline controls (both $p < .05$), but the two poisoned groups were not reliably different from each other.

It seemed possible that even though Ingestion and No-Ingestion poisoned subjects did not differ from each other in overall preference for the odorized side of the test chamber in Test 1, the two groups might have differed in how they distributed their exploration of the two compartments during the 10-min test period. However, Figure 13, showing the cumulative time spent exploring the odorized side for

Figure 12

Experiment 9: Mean percent preference for the Mentholatum odor for poisoned Ingestion and No-Ingestion subjects as well as saline controls during tests without fluids (Test 1) and with water (Tests 2 and 3). (For Test 1, numbers above bars indicate mean total sec spent exploring both test compartments. For Tests 2 and 3, numbers above bars indicate mean total ml ingested in the test compartments.)

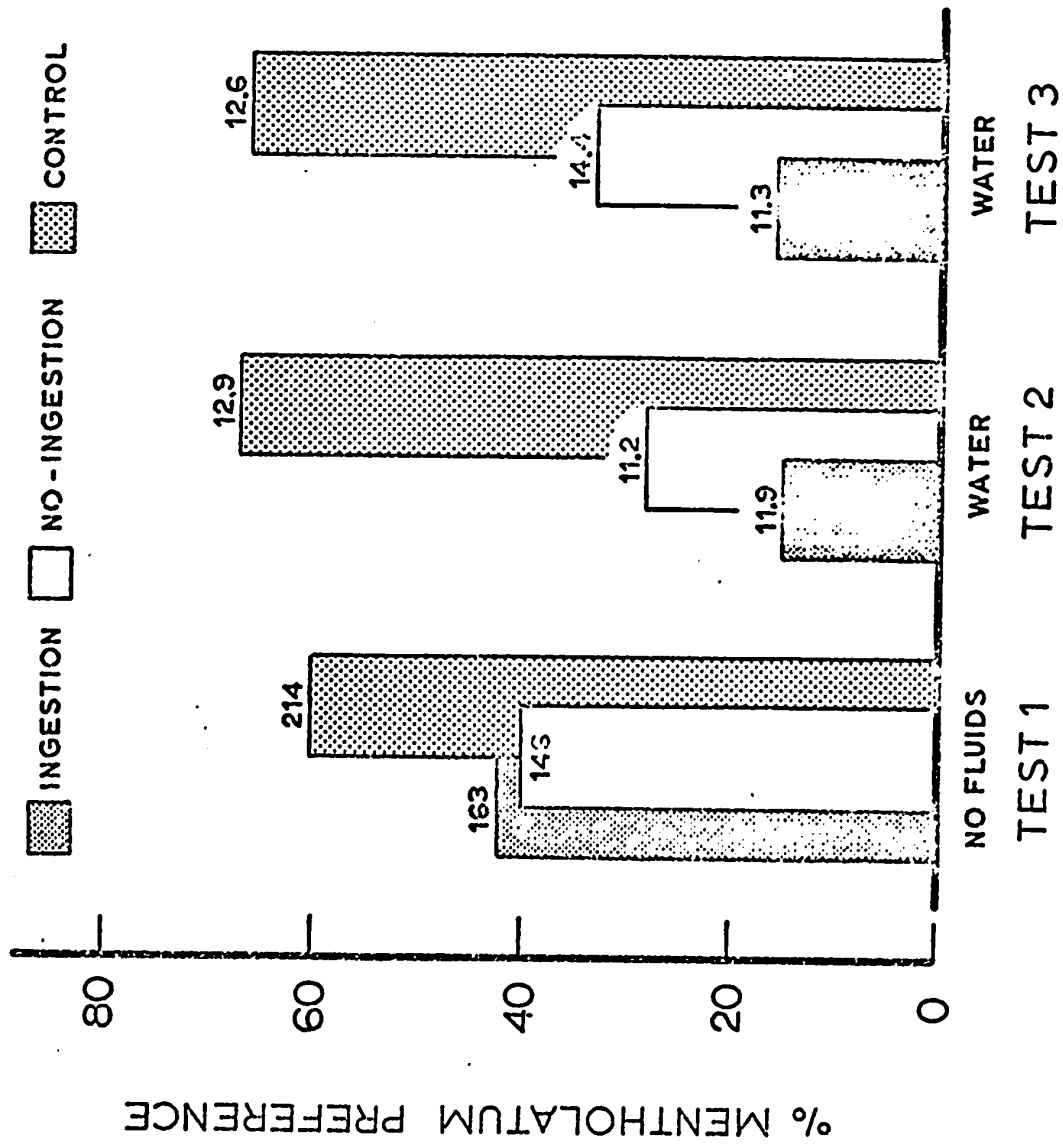


Figure 12

Figure 13

Experiment 9: Mean cumulative time spent exploring the Mentholatum test compartment during Test 1 (no fluids available) for Ingestion and No-Ingestion poisoned subjects as well as saline controls in Experiment 9.

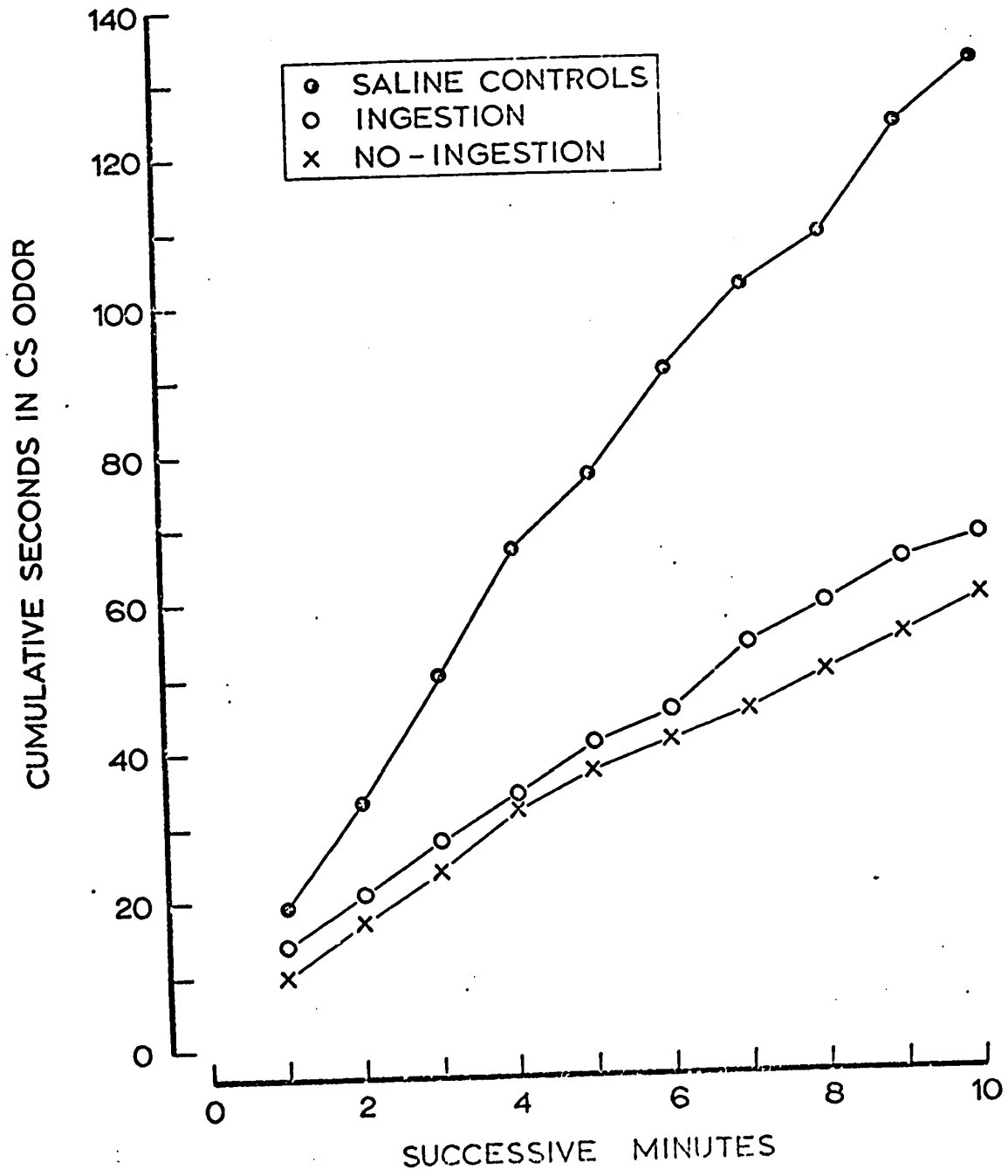


Figure 13.

successive minutes of the test period, illustrates that the two poisoned groups did not differ from each other at any time during the test session. The two poisoned groups also did not differ in the total time spent exploring the two compartments.

During Tests 2 and 3, when subjects had water available in both compartments of the choice apparatus, each of the two poisoned groups again evidenced lower preferences for the Mentholatum odor than saline controls (all $p < .01$). However, this time poisoned Ingestion subjects had lower preferences for the Mentholatum compartment than poisoned No-Ingestion subjects ($p < .05$ for Test 2 and $p < .02$ for Test 3). Thus, while the two poisoned groups showed comparable aversions to the CS odor when tested without water, ingestion subjects avoided the CS odor more than No-Ingestion subjects when tested with water. The differential effects of ingestion during conditioning were apparent only when the test task involved drinking water.

Subjects drank comparable amounts of water during Test 2 (see Figure 12), but No-Ingestion subjects drank more during Test 3 than either of the other two groups (both $p < .05$).

Discussion

The avoidance of the Mentholatum test compartment observed in the present experiment cannot be explained in terms of acquired aversions to water or other non-olfactory cues since such stimuli were identical in both test compartments.

The results of the present experiment confirm that ingestion is not necessary for odor-toxicosis learning. No-Ingestion subjects showed significant aversions to the CS odor in all three postconditioning tests.

The present results also demonstrate that odor aversions can redirect exploratory as well as ingestive behaviors. Both poisoned groups showed significant odor aversions in tests with and without drinking fluids. Previous demonstrations of odor-toxicosis learning used either the suppression of ingestion (e.g., Garcia & Koelling, 1967) or the suppression of an operant which had been previously reinforced with water (Lorden, et al., 1970) as evidence of an odor aversion. The acquired odor-suppression of exploratory behavior observed in the present study indicates that odors associated with toxicosis are aversive even if the subject is not eating or drinking.

Subjects drinking water before toxicosis during conditioning avoided the CS odor more than No-Ingestion subjects only during water-drinking tests. If the greater aversions of Ingestion subjects during tests with water were merely a result of the fact that for this group both conditioning and testing involved drinking water, No-Ingestion subjects should have evidenced greater aversions in the test without fluids since in this case both conditioning and testing did not involve ingestion. However, the two poisoned groups did not differ in the test without fluids. Thus, the present results do not fully support a generalization decrement explanation of the attenuated aversion of No-Ingestion subjects observed during the water-intake tests.

The differential effects of ingestion during odor-toxicosis conditioning may have been observed only in water-drinking tests because drinking tests of odor aversion learning are more sensitive than tests not involving ingestive behaviors. Alternatively, subjects that drank water during conditioning may have shown greater aversions when tested

with water because they associated not only the CS odor with toxicosis but also stimuli arising from an interaction between the CS odor and the flavor of water. Such stimuli, absent during the test without drinking fluids, may have been additional cues motivating the avoidance of the Mentholatum compartment for Ingestion subjects in tests with water.

Experiment 10: Comparison of Ingestion and No-Ingestion tests of odor aversion II. Water or a familiarized saccharin solution available during Ingestion tests.

Experiment 10 was designed to explore the possibility that Ingestion subjects evidenced greater odor aversions than No-Ingestion subjects when tested with water in Experiments 6, 8, and 9 because they associated not only the CS odor with toxicosis but also stimuli arising from an interaction between the CS odor and the flavor of water. It was expected that aversions conditioned to stimuli arising from an interaction of Mentholatum odor and water in Ingestion subjects would be disrupted with saccharin rather than water used as the test fluid. Therefore, Ingestion and No-Ingestion subjects were tested drinking either water or a palatable saccharin solution in the presence of Mentholatum after conditioning.

A further purpose of Experiment 10 was to see if the differential effects of ingestion on odor-toxicosis learning observed in Experiments 6, 8, and 9 could be obtained if No-Ingestion subjects are conditioned while 23-24 hr water deprived rather than just after drinking an amount of water equal to that ingested by their yoked Ingestion partners. This modification in the procedure also permitted conditioning No-Ingestion

subjects before Ingestion subjects, the reverse of the order in which subjects were run in all previous experiments.

Method

Twenty-eight rats were used. Adaptation procedures were identical to those of the previous odor-aversion experiments except that subjects were familiarized with the taste of a 0.2% saccharin sodium solution (weight/volume in tap water) prior to conditioning. The saccharin solution was substituted for water during four 30-min per day fluid access periods prior to adaptation and during the four 15-min adaptation drinking periods. Ingestion subjects ($n=14$) were allowed to drink water in the presence of the Mentholatum odor during conditioning while No-Ingestion subjects ($n=14$) had no fluids available. Unlike in previous experiments, No-Ingestion subjects also did not have fluids available just before being placed in the conditioning chambers. Ten Ingestion and No-Ingestion subjects were conditioned with lithium chloride injections as in Experiment 6 while the remaining subjects received comparable isotonic sodium chloride injections, and, as in previous experiments, subjects were given a 15-min water-drinking test in the conditioning chambers between the first and second conditioning trials.

After the second conditioning trial subjects were tested in the odor-choice apparatus as in Experiment 9. During the first test, no fluids were available in either compartment of the test chamber. During Tests 2 and 3 subjects were observed with either water or the saccharin solution available in both compartments in a counterbalanced order.

All two-group comparisons were made with the Mann-Whitney U test (two-tailed).

Results

Odor preference scores, computed for each postconditioning test session as in Experiment 9, are presented in Figure 14, with Ingestion and No-Ingestion control subjects presented as one group since they had comparable preference scores in all three tests. During Test 1, when no fluids were available in the test chamber, each of the poisoned groups had lower preferences for the Mentholatum compartment than saline controls (both $p < .02$), but the two poisoned groups were not reliably different from each other. The two groups also did not differ in the total time spent exploring the two compartments.

During Tests 2 and 3, when subjects had either saccharin or water to drink in both compartments of the odor-choice chamber, poisoned Ingestion subjects had lower preferences for the Mentholatum odor than poisoned No-Ingestion subjects when tested with water ($p < .01$). However, during tests with saccharin, the two poisoned groups were not reliably different in Mentholatum preference, even though both poisoned groups preferred drinking in the CS odor less than controls (both $p < .02$). (Preference scores computed for minutes 2, 4, 6, and 8 of the saccharin test also failed to reveal any differences between poisoned Ingestion and No-Ingestion subjects.)

The total fluid intake of Ingestion subjects was slightly greater than that of No-Ingestion subjects during the saccharin test ($p < .02$) and slightly less during the water test ($p < .02$). Ingestion subjects also drank less water than controls ($p < .02$).

Figure 14

Experiment 10: Mean percent preference for the Mentholatum odor for poisoned Ingestion and No-Ingestion subjects as well as saline controls during tests without fluids (Test 1) and with saccharin and water (Tests 2 and 3). (For Test 1, numbers above bars indicate mean total sec spent exploring both test compartments. For Tests 2 and 3, numbers above bars indicate mean total ml ingested in the test compartments.)

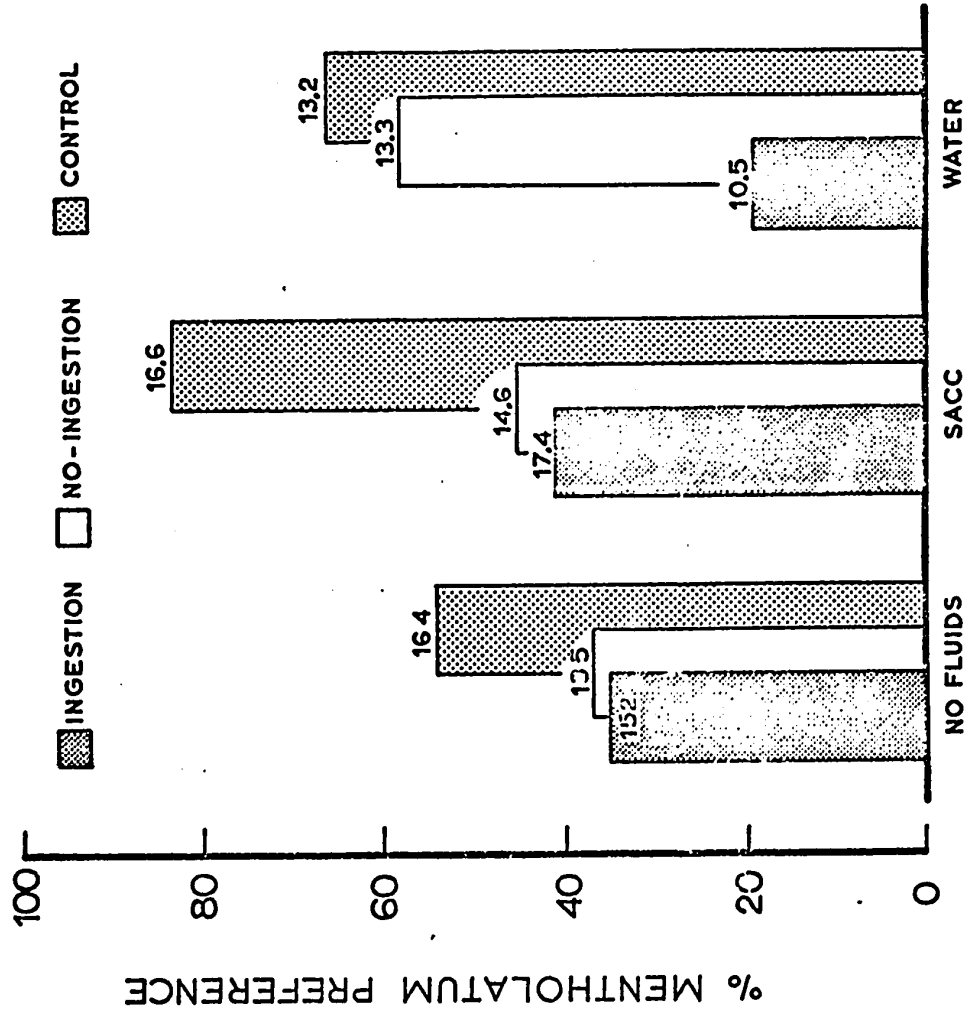


Figure 14

Discussion

As in Experiment 9, avoidance of the Mentholatum test compartment observed in the present experiment cannot be explained in terms of acquired aversions to either the flavor of the test solutions or other non-olfactory cues since such stimuli were identical in both test compartments.

Ingestion and No-Ingestion poisoned subjects evidenced comparable aversions to the CS odor in the test without drinking fluids. This finding replicates results of the no-water test in Experiment 9, and indicates that ingestion during conditioning does not influence how odors associated with toxicosis redirect exploratory behavior.

Drinking water during conditioning also did not have a differential effect on avoidance of the CS odor during the saccharin drinking test. The absence of a differential effect of ingestion on odor aversions observed in the saccharin test cannot be attributed to "ceiling" or "floor" effects since the preference scores of poisoned subjects during the saccharin test were approximately midway between a zero preference and the preference shown by saline controls.

The lack of difference between poisoned Ingestion and No-Ingestion subjects during the test without drinking fluids may have been a result of the fact that exploratory behavior tests are less sensitive to odor aversions than ingestion tests. Such an explanation, however, is inapplicable to the lack of a differential effect of ingestion observed during the saccharin drinking tests. The comparable performance of the two poisoned groups during the saccharin tests also cannot be attributed to totally ineffectual differential treatment since drinking water during conditioning was observed to facilitate avoidance of the CS odor during

the water drinking test, as in previous experiments.

The fact that drinking water during conditioning facilitated the acquired suppression of water but not saccharin intake in the presence of the CS odor suggests that Ingestion subjects associated not only the CS odor with toxicosis but also stimuli arising from an interaction between the CS odor and the flavor of water. Such water-odor interaction cues were presumably absent when subjects experienced the CS odor either in the absence of consumables or in conjunction with saccharin. Thus, No-Ingestion subjects did not have the opportunity to learn aversions to these stimuli. In addition, aversions to water-odor interaction cues acquired by Ingestion subjects could not provide additional stimuli for avoidance of the Mentholatum compartment during tests without consumables or with saccharin.

The assumption that there are water-odor interaction stimuli which can become associated with toxicosis is made in the absence of more detailed specification of the nature of these stimuli. There are no doubt many conceivable ways in which water-odor interaction cues could arise. One rather likely possibility is that the Mentholatum odor molecules become dissolved in the water while subjects are drinking and thus impart a unique flavor to the water. Thus, Ingestion subjects may associate not only the CS odor with poisoning but also the flavor of the CS odor dissolved in water. The apparent facilitatory effect of ingestion on odor-toxicosis learning therefore may be a result of an added taste aversion.

If Ingestion subjects learn aversions to the flavor of the CS odor dissolved in the ingested fluids, this taste aversion presumably would be evident even if subjects lost their sense of smell after

conditioning. Experiments 11, 12, and 13 were designed to explore this possibility.

Experiment 11: Effects of peripheral anosmia on acquired taste-aversion behavior.

In order to investigate the possibility that Mentholatum molecules can become dissolved in water and give it a unique flavor, subjects have to be tested with water in the presence of Mentholatum in a situation where their behavior cannot be motivated by an aversion to the odor of Mentholatum. Such a situation is achieved if subjects are made anosmic for the test sessions with a technique that does not disturb gustatory reception. The technique selected involved irrigation of the nasal passages with a 5% zinc sulfate solution, which produces anosmia lasting several days with minimal central damage (see Alberts and Galef, 1971). To see if this anosmia procedure disturbs taste sensitivity, the learned taste aversion behavior of subjects treated with zinc sulfate or isotonic saline after taste-toxicosis conditioning was compared using a choice test of taste aversions.

Hankins, Garcia, and Rusiniak (personal communication) recently also compared the taste-aversion learning of zinc sulfate and saline treated subjects. Although they report no effect of the anosmia treatment on taste-aversion learning, the high toxin doses and the single-stimulus test sessions used may not have permitted the detection of small differences. The present experiment also differed from that of Hankins et al. in that the anosmia treatment was administered after, rather than before, taste-toxicosis conditioning.

Method

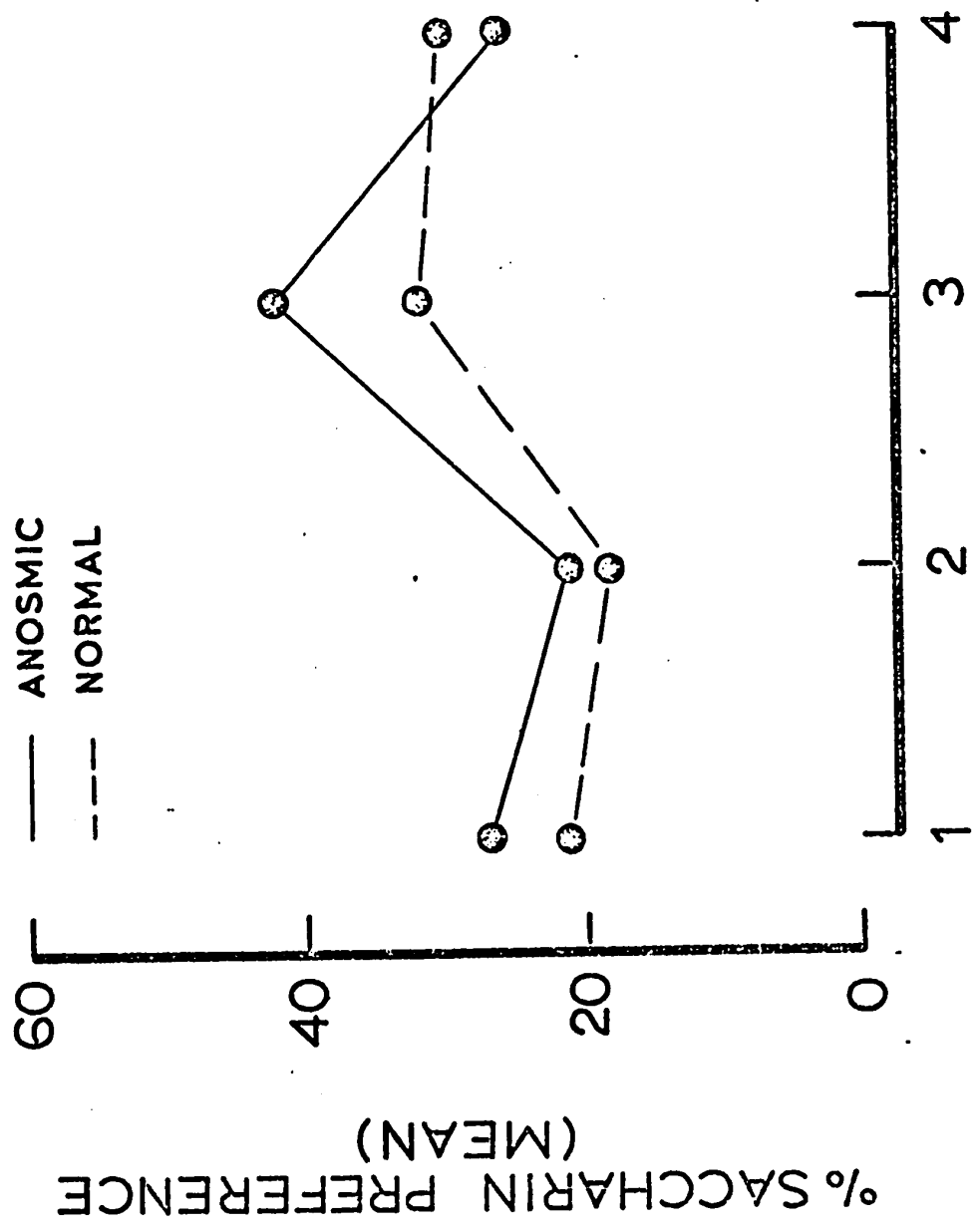
Nineteen 200-250 gm male albino Charles River rats (Quebec Breeding Farms) were individually housed with continual access to Purina Laboratory Chow and access to water limited to 45 min each day for four days. (Subjects were also adapted to handling during this period.) On the fifth day subjects were allowed to drink 8 ml of a 0.2% saccharin sodium solution (weight/volume in tap water) and were given a 0.6% body weight intraperitoneal injection of 0.12 M lithium chloride immediately afterward. Sixty to 90 min after conditioning subjects received access to water for 60 min. The next day the nasal passages of ten subjects were irrigated with 1.0 ml of a 5% zinc sulfate solution while subjects were anesthetized with ether. (See Alberts and Galef (1971) for details of the nasal irrigation procedure.) The remaining nine subjects recieved comparable treatment with physiological saline. Four, daily 1-hr saccharin-water choice tests were conducted starting the day after the nasal irrigation treatment. During each test subjects had both water and the saccharin solution simultaneously available after being forced to sample each. Preference for saccharin was measured by expressing the amount of saccharin ingested as a percentage of the total fluid intake during each test session. All statistical comparisons were made with the Mann-Whitney U test (two-tailed).

Results and Discussion

The saccharin preference scores obtained during the postconditioning test sessions are shown in Figure 15. The mean saccharin preference scores of both anosmic and normal subjects were below 50%

Figure 15

Experiment 11: Saccharin preference scores of normal and peripherally anosmic subjects after taste-toxicosis conditioning.



TESTS

Figure 15

during each test session. Since nonpoisoned subjects typically evidence 65-85% preferences during such saccharin-water choice tests (see Experiments 1, 2, 3, 4, and 5), the low saccharin preferences of subjects in the present experiment indicate that both groups had learned aversions to saccharin flavor.

The important aspect of the results, however, is not that both normal and anosmic subjects evidenced saccharin aversions but that saccharin preference scores of the two groups were not significantly different from each other during any of the test sessions (all $p > .10$). Thus, anosmia administered after taste-aversion conditioning was not found to disrupt the aversions acquired. The present results therefore confirm the findings of Hankins et al. (personal communication) that zinc sulfate treatment does not disrupt taste-aversion learning and show that peripheral anosmia does not affect taste-aversion behavior even in flavor-choice situations.

Although the zinc sulfate treatment did not affect the saccharin preference behavior observed, peripherally anosmic subjects drank slightly but significantly less fluid during the first three test sessions than the saline treated group (all $p < .05$) (see Table 1). Despite this depressed total fluid intake, however, anosmic rats drank more than a mean of 16 ml during each test session. Thus, the obtained preference ratios were not artifacts of grossly reduced intake.

Since the zinc sulfate anosmia procedure has minimal effects on taste-aversion behavior, the procedure can be used to see if subjects that drink water in the presence of the odor of Mentholatum acquire aversions to the taste of Mentholatum molecules dissolved in the water.

Table 1

Mean Total Intake (ml)

	Test 1	Test 2	Test 3	Test 4
NORMAL	20.9	22.6	22.2	22.7
ANOSMIC	16.1	18.4	19.3	21.2
<u>*p</u>	.05	.02	.05	>.10

* Reliability of the difference between normals and anosmic based on Mann-Whitney U tests (two-tailed).

Experiment 12: Effects of peripheral anosmia on
acquired odor-aversion behavior I.
Single stimulus ingestion test.

The purpose of Experiment 12 was to investigate the extent to which subjects either drinking or not drinking water during odor-toxicosis conditioning would show suppressed water intakes in the presence of the CS odor during postconditioning tests conducted after subjects had been made peripherally anosmic. The zinc sulfate nasal irrigation procedure, which does not disrupt taste-aversion behavior (see Experiment 11), was used to produce anosmia. If subjects drinking water during conditioning acquire aversions to the taste of Mentholatum, such taste aversions would presumably be evident when the subjects are tested after their olfactory epithelium has been destroyed by zinc sulfate.

Method

Thirty-four, 200-250 gm, male, albino, Charles River rats were used. Adaptation, conditioning, and test procedures were identical to those of Experiment 6, the first odor-toxicosis experiment. Subjects were assigned to Ingestion, No-Ingestion or Control groups. Six subjects in each had their nasal passages irrigated with zinc sulfate as in Experiment 11, the day after the second conditioning trial. The remaining five Ingestion and No-Ingestion subjects and six controls had their nasal passages treated with isotonic saline. Subjects received their 30-min ration of water several hours after the nasal irrigations. Response to the CS odor was assessed in a 15-min water-drinking test conducted in the conditioning chambers.

Results and Discussion

The amount of water ingested in the presence of the CS odor during the test after nasal irrigations is presented in Table 2. As expected on the basis of previous results, of the groups that had their nasal passages irrigated with isotonic saline, Ingestion poisoned subjects drank less than both No-Ingestion poisoned subjects and controls (both $p < .02$), and No-Ingestion poisoned subjects drank less than controls ($p < .01$). In contrast, none of the group differences among zinc sulfate treated subjects approached statistical significance (all $p > .30$).

Although the various zinc sulfate treated groups did not differ among each other in water intake during the test session, the control level of intake of anosmic subjects was significantly below the control level of intake of normal subjects. Nonpoisoned anosmic subjects drank a mean of 6.4 ml. less than nonpoisoned normal subjects ($p < .01$). The lack of difference among anosmic groups, therefore, may have been due to a "floor" effect introduced by the suppressed water intakes of all anosmic subjects.

Experiment 13: Effects of peripheral anosmia on acquired odor-aversion behavior II. Choice test.

Experiment 13 was designed as a replication of Experiment 12 with odor aversions assessed in the odor-choice apparatus described in Experiment 9. Since aversions in the odor-choice test are indicated by the compartment in which the subject chooses to drink rather than total amount ingested, changes in the total amount ingested would not be expected to have a large effect on the aversion behavior.

Table 2

Mean Total Intake (ml)

	INGESTION	NO-INGESTION	CONTROL
NORMAL	3.2	7.8	14.9
ANOSMIC	5.8	7.5	8.5

Method

Forty-eight, 200 to 250 gm, Charles River, male rats were used. Sixteen subjects were assigned to each of three groups -- Ingestion, No-Ingestion and Control -- with adaptation, conditioning, and test procedures identical to those of Experiment 10. The day after the second conditioning trial half the subjects in each group had their nasal epithelium destroyed by zinc sulfate irrigation (see Experiment 11) while the remaining subjects had their nasal passages irrigated with isotonic saline. The next two days, subjects were tested in a counterbalanced order without fluids or with water available in both compartments of the odor-choice chamber. Subjects that had their nasal passages irrigated with isotonic saline were then given four additional tests with water in both compartments.

Except as noted, all two-group comparisons were made with the Mann-Whitney U test (two-tailed).

Results

As in Experiments 9 and 10, response to the Mentholated compartment of the odor-choice apparatus was assessed by computing preference scores. For the test without fluids, preference scores were computed by dividing the amount of time subjects spent exploring the odorized compartment by the total time spent exploring both compartments. For the tests with water, preference scores were computed by dividing the amount ingested in the odorized compartment by the total amount ingested. These preference scores are displayed in Figure 16.

Anosmic groups did not differ in preference for the odorized compartment in either the no-fluid test (all $p > .15$) or the water

Figure 16

Experiment 13: Mean percent preference for the Mentholatum odor for anosmic (a) and normal (b) Ingestion and No-Ingestion subjects as well as saline controls during tests without fluids and with water. (For no-fluid tests numbers above bars indicate mean total sec spent exploring both tests compartments. For tests with water numbers indicate mean total ml ingested in the test compartments.)

 INGESTION
  NO - INGESTION

 CONTROL

a. ANOSMIC

b. NORMAL

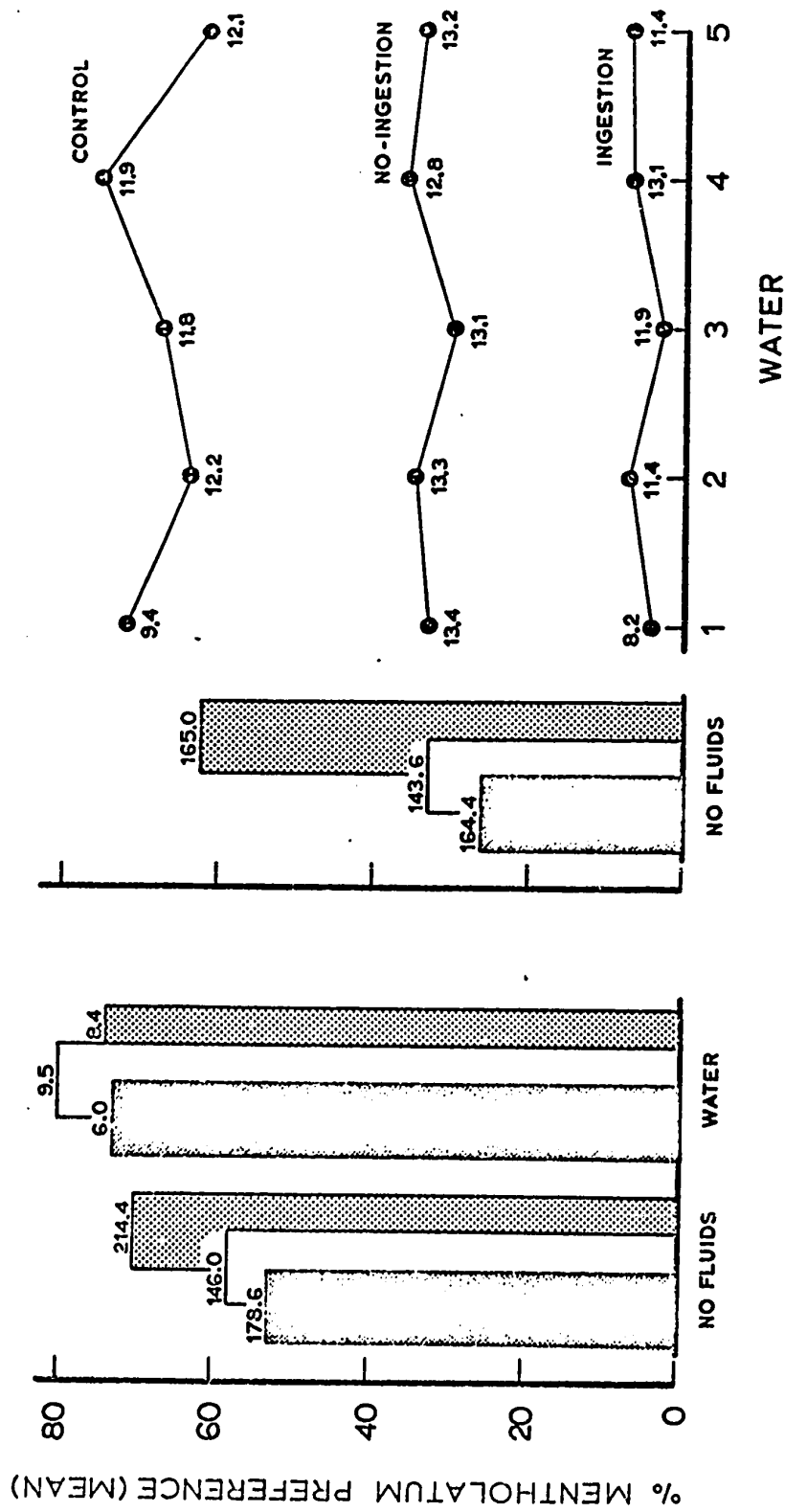


Figure 16

test (all p 's $>.50$). In contrast the two poisoned normal groups (Ingestion and No-Ingestion) preferred the odorized compartment less than normal controls both during the test without fluids (both p 's $<.01$) and the test with water (both p 's $<.01$). Ingestion and No-Ingestion normal subjects were only marginally different from each other during these first two tests ($p <.09$). However, normal Ingestion subjects had lower preferences for the odorized compartment than normal No-Ingestion subjects during each of the next four water-drinking odor-choice tests (all p 's $<.01$), as in previous experiments.

The anosmic groups did not differ in total time spent exploring the test compartments during the no-fluid test (all p 's $>.15$), but during the test with water anosmic Ingestion subjects drank slightly less than both anosmic controls ($p <.09$) and anosmic No-Ingestion subjects ($p <.04$). The normal groups also did not differ in total time spent exploring the test compartments during the no-fluid test (all p 's $>.50$). During the first test with water both normal Ingestion and Control subjects drank less than normal No-Ingestion subjects (both p 's $<.02$). Normal No-Ingestion subjects also drank more than normal Ingestion subjects during the second test with water ($p <.03$), but no other reliable differences in total fluid intake were observed among the normal groups.

As in Experiments 11 and 12, anosmic subjects drank less during the first test with water than normals ($p <.02$). However, the difference in mean water intakes between anosmic and normal subjects was only 2.3 ml in the present experiment, whereas it was 6.4 ml in Experiment 12. Such a small difference probably did not contribute

to differences in aversion behavior observed since a choice response rather than total water intake was used to assess aversions.

Anosmic and normal subjects did not differ in total time spent exploring the test compartments during the no-fluid test.

Discussion

Subjects made anosmic by zinc sulfate nasal irrigation did not evidence aversions motivated by the presence of Mentholatum in either the no-fluid or water tests. In contrast, poisoned subjects whose nasal epithelium had been treated with isotonic saline showed aversions comparable to aversions observed in previous experiments. Thus, the absence of aversions in anosmic subjects cannot be attributed to possible side-effects of the nasal irrigation procedure.

The fact that only saline-treated poisoned subjects avoided the Mentholatum-odorized compartment indicates that avoidance of the Mentholatum is based on olfactory stimulation. The absence of aversions in anosmic subjects shows that the Mentholatum is not experienced via gustatory receptors in either Ingestion or No-Ingestion subjects. The present results therefore do not support the hypothesis that Ingestion subjects acquire aversions to the flavor of Mentholatum dissolved in water. If Ingestion subjects acquire aversions to stimuli which arise from an interaction of the flavor of the solution ingested and the CS odor experienced prior to poisoning, such taste-smell interaction cues are most likely of central rather than peripheral origin.

It is interesting to note that in all three experiments, including the present one, in which the water intake of peripherally anosmic subjects was compared to the intake of subject that had their

nasal passages irrigated with isotonic saline, anosmic subjects drank less than normals. It is not clear at the present time why this result was obtained.

Summary and Conclusions

The present series of experiments was designed to evaluate the role of ingestion in odor-toxicosis learning. Subjects were poisoned after experiencing the odor of Mentholatum either in the absence of edibles or with water available. Experiment 6 showed that subjects can acquire aversions to the presence of Mentholatum whether or not they drink water during conditioning. Drinking water during odor-toxicosis conditioning, however, facilitates the acquired odor suppression of water intake. Control experiments demonstrated that aversions observed in Experiment 6 were, in fact, motivated by the Mentholatum odor as opposed to the taste of water or other non-olfactory stimuli.

The apparent facilitory effect of ingestion on odor-toxicosis learning observed in Experiment 6 was further investigated by assessing odor aversions in water and saccharin drinking tests as well as in tests involving exploratory rather than ingestive behaviors. Evidence of odor-toxicosis learning was obtained in all three types of tests. However, drinking water during odor-toxicosis conditioning was observed to facilitate avoidance of an odorized test compartment only in water drinking tests. If drinking water during odor-toxicosis conditioning had facilitated the acquisition of aversive properties by the CS odor, such a facilitory effect should have been evident in any test sensitive to odor aversions. The fact that the facilitory effect

was specific to water drinking tests suggests that drinking water during odor-toxicosis conditioning results in acquired aversions to not only the CS odor but also stimuli arising from an interaction of the CS odor and the taste of water. Experiments with peripherally anosmic subjects whose gustatory receptors remained functional indicated that the conditioned aversive odor-water interaction cues do not arise from flavors imparted to the water by the CS odor. It seems likely, therefore, that such interaction cues are central in origin.

CHAPTER 4: CONCLUSION

Taste cues are much more readily associated with toxicosis than audiovisual cues, and, unlike other types of learned aversions, taste aversions can be acquired even if the gustatory CS precedes the toxin US by several hours. Since taste cues are usually experienced only as a consequence of ingestion, in contrast to audiovisual stimulation which is often perceived in the absence of specific motivated responses, the present series of experiments was designed to evaluate the contribution of ingestive behaviors to aversion behavior motivated by toxicosis.

The experiments reported in Chapter 2 demonstrated that rats drinking a flavored solution during taste-toxicosis conditioning subsequently prefer drinking that solution less than subjects experiencing the flavor in the absence of ingestive behaviors during conditioning. Thus, ingestive behaviors were found to facilitate taste-aversion learning. However, ingestion was observed not to be necessary for learned avoidance of tastes motivated by poisoning.

Essentially the same pattern of results was observed in the odor-toxicosis learning experiments described in Chapter 3. Although ingestion was found not to be necessary for odor-aversion learning, ingestion during odor-toxicosis conditioning resulted in stronger aversions in tests involving drinking the same solution as had been ingested during training, even though there was no evidence of an enhanced aversion to the flavor of the solution used.

Because odor cues can be much more easily presented without ingestive behaviors than tastes, and the experience of odors in the absence of ingestion is not as foreign to rats as the experience of tastes without

ingestion, the mechanisms responsible for the apparent facilitory effect of ingestion or odor-aversion learning were further investigated. The pattern of results obtained suggested that subjects drinking a flavored solution during odor-toxicosis conditioning acquired aversions to not only the CS odor but also additional cues present during conditioning and postconditioning tests. Thus, the facilitory effect of ingestion on odor-toxicosis learning appears to be a result of added conditioned aversive cues stimulating the aversion behavior. The evidence presented in Chapter 3 suggests that these stimuli arise from an interaction between the taste of what is ingested and the odor experienced. Furthermore, the interaction takes place centrally as opposed to the level of the receptors.

The facilitory effect of ingestion on taste-aversion learning may also reflect acquired aversions to not only the CS -- a flavor -- but also additional cues present during conditioning and postconditioning tests, such as, for example, the interaction of taste and the proprioceptive cues of ingestion. In the case of odor-aversion learning, the additional stimuli to which ingestion subjects presumably learned aversions could be removed by either removing consumables or by sufficiently altering the flavor of what was ingested. In taste-aversion learning, however, the hypothesized added conditioned aversive stimuli can be removed only by preventing ingestive behaviors, since changing the flavor of what is ingestion would also alter the "primary" taste CS involved.

It should be noted that the particular account of the role of ingestion in aversion learning favored in the present discussion is

stated in terms of stimuli experienced as a consequence of ingestion rather than in terms of motivation for engaging in the ingestive behaviors. The added stimuli Ingestion subjects presumably associate with poisoning would be expected to be unaffected by the procedures used to bring subjects in contact with these stimuli. Thus, if the present account is true the facilitory effect of ingestion on taste and odor aversion learning should obtain even if ingestion is motivated by avoidance of electric shock, for example, rather than thirst-reduction, provided the same pattern of ingestion can be produced and the shock schedule has no deleterious effects on aversion learning motivated by toxicosis.

Evidence that motivational factors were not responsible for the effects of ingestion observed in taste-aversion learning was provided by the demonstration that deprived and watered rats acquire comparable taste aversions if they both drink something during conditioning (Experiment 3). Furthermore, the facilitory effect of ingestion on odor-aversion learning was observed whether or not Ingestion subjects were more water deprived than No Ingestion subjects during conditioning. (In Experiments 6, 8, 9, and 12 Ingestion subjects were 23 to 24 hours water deprived when placed in the conditioning chambers, whereas No-Ingestion subjects had just had access to water. In Experiments 10 and 13 Ingestion and No-Ingestion subjects were equally water deprived when placed in the conditioning chambers.)

Shettleworth (1972) recently found that ingestion-associated stimuli can also influence the course of aversion learning in domestic chicks conditioned with foot shock. Ingestion during conditioning led

to the association of visual cues with shock whereas no-ingestion led to the association of auditory cues with shock. Whether the mechanisms of this effect are similar to the ingestion effects explored in the present experiments remains to be investigated.

With the exception of Shettleworth (1972), previous experiments on aversion learning treated ingestive behaviors during conditioning as simply a convenient technique for bringing subjects in contact with the CS taste or odor. The results of the present series of experiments indicate that other consequences of ingestion must also be considered. Ingestion not only brings subjects in contact with taste and food-associated olfactory cues but also facilitates the conditioning aversion behavior observed in the ingestion tests typically used in aversion experiments.

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