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The pairing of a novel flavor with an aversive event, such as nausea, often results in subsequent avoidance of the novel flavor. Termed taste aversion (TA) learning, this phenomenon has been the focus of extensive research. Findings suggest an adaptive-evolutionary foundation underlying the robustness, generality, and durability of TA learning. Investigations of factors influencing its strength and occurrence in addition to biological bases have clarified many issues surrounding eating and drinking behavior. However, a possible genetic component has received little attention in the literature of the field.

In order to examine a potential genetic contribution to the conditionability of an avoidance to taste, an experimental design was established. TA learning was assessed in the offspring of rats selectively bred on the basis of efficiency in TA acquisition. Although some evidence was found supporting an impact of selective breeding, additional results remain ambiguous. Continued line development from the distinct groups which emerged in this study may yet enhance understanding of the basic phenomenon of taste aversion and its genetic components.

THE DEVELOPMENT OF STRAINS OF TASTE AVERSION PRONE AND TASTE AVERSION RESISTANT ANIMALS

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CHAPTER 1

Introduction

The pairing of a novel flavor with an aversive event, such as nausea, often results in subsequent avoidance of the novel flavor. This phenomenon, called taste aversion learning, is typically explained via a classical conditioning model. Accordingly, an initially neutral stimulus (the conditioned stimulus flavor or CS) is paired with the unconditioned stimulus (US) that reflexively elicits illness (the unconditioned response or UR). As a result of this CS-US pairing, the CS, when presented alone, elicits avoidance of the previously novel flavor. The occurrence of this conditioned avoidance response reflects the development of a taste aversion (TA).

Garcia and colleagues are recognized for introducing TA learning as an organized research focus in 1966. In the first of two classic experiments with rats, Garcia and Koelling (1966) demonstrated that gustatory CSs but not auditory and visual CSs produced conditioned avoidance reactions when paired with nausea induced by radiation or lithium chloride. Conversely, audiovisual CSs but not gustatory CSs, when paired with electric shock resulted in conditioned aversions. This effect, cue-to-consequence specificity, was inconsistent with the equipotentiality assumption of accepted learning theory. In essence, this premise of equivalence states that all stimuli and responses

are equally associable (i.e., any perceptible stimulus can signal reward or punishment).

The latter of these two seminal works presented yet another contradiction of the traditional laws of learning. Garcia, Ervin, and Koelling (1966) showed long-delay learning in that conditioning occurred even when UR onset followed CS ingestion by an interval of 75 minutes. Associations had been assumed to occur only when stimuli and response were separated by about .5 second, that is, in close temporal contiguity (Kimble, 1961).

Because these studies were seen as challenges to the principles of equipotentiality and temporal contiguity within accepted learning theory, they stimulated an abundance of creative commentary and research in attempts to explain, refute, or incorporate the findings (Rozin, 1977). In less than 20 years, a bibliography of 1,373 papers published on TA learning was amassed (Riley & Tuck, 1985).

This quickly expanding body of literature provided overwhelming support for Garcia's original observations and illustrated both nutritional wisdom in animals and differences in innate preferences for consummatory cues (Zahorik & Houpt, 1977). In doing so, it contributed to the development of divergent perspectives on learning theory, each encompassing TA learning. For example, Seligman (1970) suggested a subset of the laws of learning based on a continuum of species-specific preparedness, Rozin and Kalat

(1971) described a theory of adaptive specialization, and Rozin (1976) proposed the evolution of phylogenetic differences in accessibility of learning abilities. More recently, a reformulation of affective processing of taste as US and nausea as feedback (FB) based on biological adaptation was advanced by Garcia (1989). Evidenced in each of these theories is an adaptive-evolutionary foundation. As Garcia (1989) succinctly states, "In order to survive and reproduce, all organisms evolved coping mechanisms for obtaining nutrients and protective mechanisms to keep from becoming nutrients" (p. 67). This perspective has enhanced the understanding of feeding and drinking behavior across situations and species.

Gustavson (1977) suggests, "The most striking aspect of a comparative overview of taste aversion conditioning is the consistency of the results obtained across species with the basic conditioning procedure" (p. 23). Also provided by Gustavson are details of studies investigating such avoidance learning in more than 30 species in addition to humans. Garb and Stunkard (1974), Logue (1985), and Rozin (1986) concur: TA conditioning in humans is apparently very similar to that in other species, and it occurs under natural conditions.

Whether established under experimental conditions or in natural habitat, TAs have been shown to be remarkably resistant to extinction. Rats have maintained the original

intensity of a conditioned aversion over extinction trials extending 50 to 60 days (Elkins, 1973, 1974). Among humans, De Silva and Rachman (1987) and Garb and Stunkard (1974) reported food aversions acquired in childhood which spanned 50 years.

Having established the robustness, generality, and durability of the basic phenomenon, subsequent research examined factors which influence the strength and/or occurrence of TA learning. For example, long-delay learning has been further explored. Reports indicate successful TAs have been induced in rats with CS-US delays of 24 hours (Estcorn & Stephens, 1973). Yet a delay of reinforcement gradient is apparent; the intensity of predictable conditioning, as measured by consumption of the target substance, diminishes as the CS-US interval is lengthened (Kalat & Rozin, 1971; Smith & Roll, 1967). During the delay between presentation of CS and US, an animal may gradually learn the taste is safe (Kalat & Rozin, 1973) or unrelated to illness (Kalat, 1977).

In addition to application in explaining the delay of reinforcement gradient, the concepts of learned safety (Best & Barker, 1977; Kalat & Rozin, 1973) and learned noncorrelation (Kalat, 1977) have been employed to describe attenuation of conditioning by familiarity with the to-be-conditioned taste. CS exposure which is not followed by illness prior to conditioning (pre-exposure) interferes

with aversion learning in subsequent pairings of the taste with toxicosis. Although reduction in the associability of the familiar stimulus as the result of pre-exposure, termed latent inhibition (Lubow, 1973), has been noted, strong TAs to familiar stimuli can be acquired with repeated conditioning trials and discrimination training (e.g., Elkins, 1974; Riley, Jacobs, & Mastropaolo, 1983).

Extensions of investigations into CS familiarity and intensity have explored the influence of presenting compound CSs. Best, Best, and Henggeler (1977) discuss and cite evidence for the overshadowing phenomenon: the more highly salient of two CSs introduced on conditioning assumes greater associative strength with the US, while the less salient cue shows weaker conditioning. The less salient cue may even be prevented or blocked from acquiring conditioned properties. Overshadowing of one taste by yet another taste has been demonstrated (Cannon et al., 1985; Revusky, 1971). Bouton and Whiting (1982) similarly showed that taste overshadowed odor cues.

However, seemingly contradictory evidence supports facilitation of conditioning to CSs presented in conjunction with taste. Known as potentiation, the effect was observed by Bouton, Dunlap, and Swartzentruber (1987) and Davis, Best, and Grover (1988) in studies employing taste-taste compounds. Potentiation by taste has also been demonstrated in distal cues classified as olfactory (Coburn, Garcia,

Kiefer, & Rusiniak, 1984), visual (Galef & Osborne, 1978), auditory (Ellins, Cramer, & Whitmore, 1985), and environmental (Best, Batson, Meachum, Brown, & Ringer, 1985) but only under suitable circumstances such as spatial and temporal contiguity. The establishment of multiple associations between the target and potentiating stimuli and between each CS and the US offers a plausible explanation for this effect (Davis et al., 1990; Durlach & Rescorla, 1980). Potentiation characterized as the summation of these associations, Bouton et al. (1987) suggest, "may depend on the relationship between the concentration of the target and that of the potentiating taste...under conditions that facilitate perception of the compound as a unit" (p. 437; see also Rescorla, 1981). The outcomes of a series of experiments by Davis et al. (1988) support the theory of relative salience within the perceptual integration interpretation.

The precise circumstances under which a target stimulus might be overshadowed or alternatively perceived as a feature of another stimulus and thus potentiated remain unclear. Garcia (1989) declares no paradox between overshadowing and potentiation exists when viewed in the "CS-US-FB" conceptualization. In this paradigm, taste is returned to the Pavlovian function of US, and distal cues, if presented, are CSs. Nausea serves as internal feedback (FB) producing a hedonic shift in US taste value evidenced

by development of an avoidance response. In the case of overshadowing, compound CSs (or compound USs) compete for association with one FB. Only one stimulus of the competing pair, the more salient member, acquires aversive strength; association of the less salient member with the FB is overshadowed. Potentiation is similarly described as a process of classical conditioning; a single CS introduced to the US-FB pairing gains associative properties.

In the traditional CS-US model of TA learning, taste, the stimulus to be conditioned, is not the only variable which impacts learning. The character of the US also influences TA acquisition. Since Garcia and Koelling's (1966) work demonstrated the cue-to-consequence effect, the role of internal distress has received much attention. Although nausea plays an important role in effective TA conditioning in humans (Pelchat & Rozin, 1982) and may be sufficient for TA development, it does not seem to be necessary (Gamzu, 1977; Gamzu, Vincent, & Boff, 1985). Garcia, Lasiter, Bermudez-Rattoni, and Deems (1985) elaborate:

> Flavor (odor and taste) aversions are the most sensitive behavioral index of prior emetic toxicosis in an ascending series of psychological reactions including anorexia, nausea, vertigo, and finally emesis. Therefore, a food aversion is often manifested in the absence of any other behavioral corroboration

> [in addition to avoidance]. Aversions can be induced by any agent that, at a higher dose, will produce emetic malaise. Thus, aversions are also produced by vestibular stimulation, by intense pain and emotion, or by verbal suggestion in humans. (p. 13)

That TAs have been elicited by verbal suggestion implies conscious mediation or intentionality may be a factor in conditioned avoidance. Indeed, even "thinking about [conditioned flavor aversions] elicits reports of nausea and facial expressions of loathing in humans" (Garcia, 1989, p. 48; see also Elkins, 1984). However, cognitive processing is not an essential component of TA learning. Supporting the earlier findings of Roll and Smith (1972), Bermudez-Rattoni, Forthman, Sanchez, Perez, and Garcia (1988) demonstrated single-trial TA acquisition in rats subjected to the influence of anesthesia and sedative and analgesic agents before induction of nausea. Aversions were also generated among rats in which Buresova and Bures (1973) induced chemically depressed electrocortical activity in one or both hemispheres. Among humans, food aversions have been acquired in the absence of any known instance of related illness. Such aversions have persisted for decades in spite of some degree of certainty that the aversive food was not the causal factor (De Silva & Rachman, 1987; Garb & Stunkard, 1974; Logue, 1985). These studies show effective conditioning is neither contingent on cortical participation

nor mediated by cognitions.

Such early experiments focusing on the strength and occurrence of learned TAs led to the search for neurological and physiological mechanisms of aversion conditioning. Gamzu et al. (1985) offered summaries of TA-inducing pharmacological agents and the neurotransmitter systems which they impact. Further investigations (cf. Bardo & Valone, 1994; De Beun, Rijk, & Broekkamp, 1993; Mark, Blander, & Hoebel, 1991) have expanded this research base. Findings provide support for independent systems for processing diverse stimuli. Garcia et al. (1985) incorporate the adaptive-evolutionary approach in describing these systems:

> The organization of the vertebrate brain reveals two specialized defensive systems that have evolved in response to selection pressures inherent in the food chain. To protect its skin from predator attack, the vertebrate selectively associates exteroceptive stimuli with peripheral insults. To protect its gut from toxic foods, the animal selectively associates taste with delayed illness. (p. 10)

The skin defense system is represented in distinct neural pathways where somatosensory and auditory information converge, whereas pathways for gustatory and gastrointestinal information constitute the gut defense system. Accessing both systems are visual and olfactory

pathways; separating the two systems are sensory gates, reciprocal mechanisms controlling excitation/inhibition. Activation of these gated pathways in a specific temporal sequence, subsequent internal feedback (e.g., nausea or pain), and the resulting negative shift in stimulus value from palatable to unpalatable account for aversion learning and specificity of cue to consequence (Garcia, 1989; Garcia et al., 1985).

Although biological research has advanced the understanding of these underlying mechanisms, the influence of genetic contributions to TA conditioning remains open to exploration. In response to a call for examination of such factors (Elkins, 1973), bidirectional selective breeding of Sprague-Dawley derived rats based on TA conditionability was begun in 1977 (Elkins, 1991). Diverging strains of taste aversion prone (TAP) and taste aversion resistant (TAR) animals have been produced. In conjunction with the goal of clarifying TA phenomena, Elkins, Walters, and Orr (1992) envision benefits of an applied nature specifically impacting the aversion-based treatment of alcoholism. These benefits may include:

> early identification and education of humans who are naturally resistant to [acquiring alcohol-related aversions]..., development of biologically based criteria for determining those consummatory aversion prone alcoholics who may be predisposed to benefit from

> emetic therapy treatments..., [and] pharmacological strategies to enhance the emetic therapy conditionability of naturally [conditioned aversion] resistant alcoholics. (p. 933)

This program of research has already yielded significant results. Beginning with the second selected generation (S-2) and continuing through the 22nd generation (S-22), TAP and TAR lines of rats have differed reliably in TA learning with low within-line variability (Hobbs, Walters, Shealy, & Elkins, 1993). As a control procedure, pseudoconditioning has been implemented. Treatment was varied for the control groups only through delivery of water in lieu of a saccharin solution CS or through injection with physiological saline substituted for cyclophosphamide as the US. Subjects of these groups representing the divergent lines have shown no difference in TA acquisition. Neither TAP nor TAR pseudoconditioned animals formed aversions to saccharin as reflected in preference testing following conditioning. These findings support a conditioning interpretation of the strain deviations in TA propensities (Elkins et al., 1992; Hobbs et al., 1993).

Although TA learning differs between the strains, the TAP and TAR animals do not differ with regard to other learning abilities. For example, shock-motivated environmental avoidance (SMEA) responses have been examined. In this task, efficient SMEA acquisition is measured by

prolonged avoidance of a shuttlebox compartment where the subject previously experienced electric shock. Elkins et al. (1992) reported no strain divergence in SMEA learning through the S-22 generation. "The attainment of TA strain divergence without comparable SMEA separation...," Elkins (1986) states, "is consistent with the neuroanatomical diversity hypothesis [of distinct skin and gut defense systems] and, by extension, with the hypothesis that different genes subserve TA and SMEA learning mechanisms" (p. 123).

Instrumental conditioning procedures have also been implemented. TAP and TAR lines have been found to be equivalent learners of food reinforced foraging in a radial arm maze (Hobbs et al., 1993) and barpressing under varying schedules of reinforcement (Hobbs & Elkins, 1983). These studies provide evidence that selective breeding has influenced TA conditionability without impacting learning ability specific to maze and barpressing performance.

Noting that the TAP and TAR animals were avid predators of live crickets, Elkins, Gerardot, and Hobbs (1989) reported maintenance of representative strain differences when cyclophosphamide injection followed cricket ingestion. Thus, conditionability is not restricted to the use of a saccharin CS.

Similar findings have been noted when alternative methods of US induction were employed. Characteristic strain

differences were found with the use of rotational stimulation (Elkins & Harrison, 1983; Elkins, Walters, Harrison, & Albrecht, 1990), lithium chloride (Elkins et al., 1992), emetine hydrochloride (Elkins & Walters, 1990; Elkins et al., 1992), ethanol (Elkins & Walls, 1988; Elkins, Walters, Orr, Kolbe, Ritch, et al., 1991; Elkins et al., 1992), and cocaine hydrochloride (Elkins, Walters, Orr, Kolbe, Westbrook, et al., 1991). Thus, US specificity is not a factor in the development of the TAP and TAR strains.

Preliminary investigation of neurochemical correlations with TA conditionability have begun. Orr, Walters, Carl, and Elkins (1993) showed higher levels of serotonin and lower levels of norepinephrine and lysine in whole-brain examinations of TAP as compared to TAR animals. On the other hand, between-lines similarity in muscarinic receptor densities and acetylcholinesterase activity in tissues of the frontal cortex, striatum, amygdala, hippocampus, and pons was reported by Aronstam, Elkins, and Walters (1990). Implicated then in genetic bases of TA conditionability are roles of specific monoamines and at least one amino acid; involvement of cholinergic systems remains a possibility. The precise relevance of these findings awaits pharmacological manipulations and further research involving specific brain regions.

The work of Elkins and colleagues in developing and examining these divergent rat strains remains unique in

reports of taste aversion research. The need for replication is recognized. In the hope of establishing a foundation from which an ongoing program of research will be formulated, the present study was specifically designed to test TA learning in the offspring of rats selectively bred for TA conditionability. Such a program holds potential for contributing to the understanding of genetic factors influencing conditionability of aversions. Studies of the similarities and differences in well-established phenomena such as latent inhibition, resistance to extinction, and potentiation/overshadowing in these animals, as compared to other selected and nonselected strains, will more clearly delineate those factors.

Exploring the possibility of overriding inherent TA proneness and resistance would also be interesting. For example, early dietary manipulations influence the ability of an animal to use specific cues to avoid poisoning later in life. Whereas rat pups are superior to adult rats in associating odor with illness, mature animals more effectively utilize taste cues to modulate feeding. "When rats are given 'enriched' experience with odor and taste after weaning," Garcia (1989) states, "their adult capacity to utilize odor in response to delayed nausea is vastly improved" (p. 67). Similarly, exposure to a variety of tastes during development might enhance conditionability of aversions to novel tastes at maturity among rats resistant

to acquiring TAs.

The ability to effect a negative impact on conditioning, thus overriding a genetic predisposition toward TA proneness, might also be tested. Social variables, for example, inhibit the association of taste with toxicity. In a series of experiments involving rats, Galef (1989) allowed subjects to interact with conspecifics that had consumed a novel, palatable target taste. Subsequent attenuation of TA conditioning of the subjects to that taste was reported. Comparable impairment of acquisition among rats bred for TA learning efficiency might be observed.

Finally, the heuristic value of such studies must be recognized. Examination of genetic contributions to aversion conditioning will facilitate and guide the development of further research. An established program of this nature will serve as a catalyst generating formulation of new questions and strategies for investigating the basic phenomenon of taste aversion.

CHAPTER 2

Method

In order to test for a potential genetic contribution to the conditionability of an avoidance to taste, a two-phase design was established. Phase 1 consisted of determination of TA learning propensity among experimentally naive rats and, based on the findings, selective breeding of these animals. Offspring of the Phase 1 rats were designated either taste aversion prone or taste aversion resistant on the basis of breeding and were examined and compared for TA learning efficiency during Phase 2.

Participants

Experimentally naive rats obtained from the Holtzman Company (Madison, WI) and the first offspring generation of these animals participated in the present project. Housed individually in suspended wire-mesh cages, the animals were allowed free access to lab chow and, except as noted, tap water. The colony room was maintained at 74 ± 4°F under a 0800 CST onset and 2000 CST offset light-dark cycle.

General Methodology

Each conditioning and testing sequence for the parent generation (Phase 1) and offspring (Phase 2) encompassed 13 days. The general procedures are described at this point; modifications and specifics follow.

A fluid deprivation schedule was implemented at the inception of each phase. On Day 1 water bottles were removed

from the home cages. For each of six days thereafter, water was made accessible for a 20-min period in standard drinking bottles. All procedures began at the same hour daily.

Conditioning was administered in the following manner on Day 8. First, all animals received 10-min exposure to a novel saccharin solution (.15% w/v) dispensed from graduated centrifuge tubes. Fifteen min following fluid removal, each animal received an intraperitoneal injection of lithium chloride (12 mg/kg of 0.15 M solution).

Four rest days during which water was made available for 20-min daily followed conditioning. On Day 13 (testing) subjects received 20-min access to the saccharin solution. Measures of saccharin consumption were recorded.

All participants were weighed at the beginning of their respective experimental phase and on Days 4, 7, 10, and 12. Experimental procedures were executed between the hours of 1000 and 1500 CST.

Phase 1

Participants. Sixteen male and 16 female rats comprised the subject pool for Phase 1. These animals were approximately 60 days old when they were received from the supplier and 90 days old when the research was initiated.

Procedures. Conditioning and testing procedures as detailed above were employed. Measures of the volume of saccharin consumption (ml) on conditioning (Day 8) and testing (Day 13) were compared. Selective matings of the

three pairs of animals that consumed the least saccharin on testing and the three pairs of animals that consumed the most saccharin on testing produced the generation studied in Phase 2.

Phase 2

Participants. The 64 surviving offspring of the selectively bred Phase 1 rats served as participants in Phase 2. One litter of animals was stillborn, and an additional animal did not complete the experiment due to illness. Groups were identified on the basis of breeding. Offspring produced by the pairing of Phase 1 animals showing the strongest conditioning (least consumption on testing) and, alternatively, of those Phase 1 animals exhibiting the weakest conditioning (most consumption on testing) were designated taste aversion-prone (TAP) and taste aversion-resistant (TAR), respectively. These rats (15 males and 28 females of Group TAP, 10 males and 11 females of Group TAR) were approximately 90 days old when Phase 2 was initiated.

Procedures. Methodology of Phase 2 followed the established conditioning and testing procedures. Saccharin consumption on conditioning and testing was measured to the nearest .01 gram on an electronic digital scale (Acculab Model #V-200).

CHAPTER 3

Results

Prior to analysis the fluid consumption data of the Phase 1 animals were converted from volume (ml) to mass (g) to facilitate comparison of the two phases. An alpha level of .05 was utilized in all statistical tests.

An analysis of variance (ANOVA) was conducted to examine the dependent variable, saccharin consumption. Data recorded in both phases of the experiment were included to enable comparison of the offspring groups in addition to contrasts across the generations. Independent variables were represented in measures of Group (Phase 1, Phase 2: TAP, or Phase 2: TAR) and Time (conditioning and testing). While no interaction of Group X Time was found, significantly lower scores were noted at testing, as compared to conditioning, $F(1, 186) = 359.36$, $p < .001$, reflecting TA formation. A significant difference by Group was also found $F(2, 186) = 6.04$, $p < .01$. Table 1 presents means, standard deviations, and variances by Group and Time.

The test used in the previous analysis, ANOVA, is robust with respect to minor violation of the assumption of homogeneity of error variance (Kirk, 1968, chap. 2). However, visual inspection of the raw and transformed data suggests strong violation of this assumption. In view of this concern, alternative nonparametric analyses were utilized, initially to explore the data of each phase and

Table 1

Phases 1 and 2 Saccharin Consumption in Grams by Group and Time

	M	SD	V
Phase 1 (n = 32)			
Conditioning	8.74	2.72	7.42
Testing	1.14	1.71	2.92
Phase 2: TAP (n = 43)			
Conditioning	8.41	3.32	11.01
Testing	1.57	1.37	1.89
Phase 2: TAR (n = 21)			
Conditioning	6.51	3.09	9.55
Testing	0.54	0.28	0.08

then to contrast Phase 1 and Phase 2 measures. The Mann-Whitney U test allowed comparison of consumption scores; for examination of variability, Levene's F test was chosen on the basis of robustness and power (Glass, 1970).

Phase 1

In order to determine the occurrence of TA conditioning of the parent generation of participants, a Mann-Whitney U test was completed. The amount of saccharin solution consumed dropped significantly from conditioning to testing, $U = 19.50$, $p < .001$. Establishment of an aversion to the taste of saccharin following conditioning was clearly indicated. Levene's test for equal variances, $F(1, 62) = 7.28$, $p < .01$, showed significantly greater variability at conditioning as compared to testing among the Phase 1 animals.

Phase 2

Phase 2 consumption scores at conditioning and testing were examined to ascertain TA acquisition. Following these tests, group measures were compared at testing and then at conditioning. Finally, a difference score for each animal reflecting the change in consumption from conditioning to testing was calculated; TAP and TAR difference scores were contrasted.

Mann-Whitney U tests showed significantly less saccharin was consumed at testing as compared to conditioning by Groups TAP and TAR, $U = 41.00$, $p < .001$, and

U = 2.00, p < .001, respectively. These results indicate TAs were acquired by both Phase 2 groups. Significance was attained in comparisons of variances at testing and conditioning for Group TAP, Levene's $F(1, 84) = 32.55$, p < .001, and for Group TAR, Levene's $F(1, 40) = 31.82$, p < .001; both Phase 2 groups varied more at conditioning than at testing.

A test of group consumption scores at testing yielded significance, Mann-Whitney $U = 647.00$, $p < .01$. Group TAR, the offspring of the animals which demonstrated the least efficiency in TA acquisition in Phase 1, consumed significantly less saccharin solution than Group TAP, rats selectively bred from the Phase 1 animals which most readily learned to avoid the target taste. Significant heterogeneity of variance in TAP and TAR testing scores was found, Levene's $F(1, 62) = 26.87$, $p < .001$, reflecting greater variability among TAP animals. In addition to lower consumption and variability at testing, Group TAR drank significantly less saccharin at conditioning than did Group TAP, Mann-Whitney $U = 314.00$, $p < .05$, although no divergence in variability was seen.

A final test of Phase 2 data was conducted to examine the magnitude of the decrease in consumption observed following conditioning. For each animal, the difference between measures of saccharin intake at conditioning and at testing was calculated. Comparison of TAP and TAR difference

scores revealed no disparity between the groups.

Phase 1 vs. Phase 2

The conditioning and testing scores of each Phase 2 group, TAP and TAR, were contrasted with the data of the Phase 1 rats. Through application of the Mann-Whitney test, a significant difference was found in lower drinking scores of Group TAP than Phase 1 at testing, $U = 453.00$, $p < .02$. Divergence was also noted between Group TAR and Phase 1 measures. As compared to the parent generation, Group TAR drank significantly less at conditioning, Mann-Whitney $U = 462.00$, $p < .05$, and showed significantly less variability at testing, Levene's $F(1, 51) = 14.58$, $p < .001$. In order to contrast the Phase 1 change in drinking pattern from conditioning to testing with the amount of decrease observed in each Phase 2 group, a difference score was calculated for each Phase 1 animal. Comparisons of the difference scores of Phase 1 and each Phase 2 group showed no dissimilarity.

CHAPTER 4

Discussion

The present study was designed to assess TA learning in the offspring of rats selectively bred for TA conditionability. More specifically, Phase 1 animals were conditioned and tested; these participants were then paired for mating on the basis of TA learning efficiency. In Phase 2, offspring of the Phase 1 animals were examined for conditionability of an aversive response to taste.

Consistent with previous reports of selective breeding of rats for TA conditionability (Elkins, 1986; see also Elkins & Harrison, 1983), the results of this experiment reflect conditioning of avoidance to saccharin in parent and offspring generations. Phase 1 animals, as well as TAP and TAR animals in Phase 2, demonstrated association of the target taste with toxicosis and showed greater within-group homogeneity at testing. Furthermore, among the Phase 2 rats selectively bred for TA resistance or proneness, two distinct groups emerged. While no difference in the volume of drinking at testing was noted between Group TAR and the rats of Phase 1, the finding that Group TAP consumed less solution at testing than Phase 1 animals suggests an impact of breeding for TA learning efficiency. However, unexpected was the outcome of a comparison of the Phase 2 groups; weak TA learners produced offspring (Group TAR) which drank significantly less solution of the target taste at testing

than the offspring of breeders shown to be stronger in conditionability (Group TAP).

Among the alternatives for explaining this anomaly is a reversal of TA learning efficiency in the TAR animals. This group bred for TA resistance appears to exhibit a greater propensity for acquiring an aversion to taste than the animals bred for TA proneness. Unidentified disparity between the Sprague-Dawley strain of rats participating in the prototype selective breeding program (see Elkins & Harrison, 1983) and the Holtzman strain of this replication could contribute to the apparent contradiction in the results obtained. Although strain differences in genetic contributions to the intensity of learned avoidance remain to be explored, other between-strain factors influencing the development of aversions have been examined. Following an investigation of TA learning among seven inbred strains of rats, Cannon, Leeka, and Block (1994) reported, "no evidence [suggesting diversity in] general ability to form taste-toxicosis associations" (p. 802). Yet support has been offered for strain variations in CS palatability and, consequently, initial consumption which impact the acquisition of aversions (Cannon & Carrell, 1987; Cannon et al., 1994).

The hypothesis that a CS taste for a specific strain may be more or less aversive and that resulting consumption differences on the initial drinking episode may confound the

results of TA learning investigations suggests another explanation of the outcome of this experiment. Indeed, comparisons of Phase 1 and Phase 2 measures and of the Phase 2 group data provide support for an interpretation based on disparity between the generations and groups before the pairing of the target taste with illness.

A significant group difference in the rats bred from weak TA learners (Group TAR) was evidenced by lower consumption at conditioning than either Phase 1 animals or Group TAP. Group TAR in contrast to the other participants (a) may have found the taste of saccharin less palatable, (b) may be more likely to avoid any novel substance, or (c) may differ by some other mechanism which suppressed consumption of the target taste when first encountered and perhaps again at testing.

Group TAR might be expected to exhibit greater homogeneity in all comparisons to the other animals in the study. The findings of significantly less variance in the testing scores, but not conditioning scores, of Group TAR than Phase 1 or TAP animals is inconclusive. Less variability would be anticipated in groups of animals bred for a specific TA propensity than in the generation from which their parents were selected. Support for this interpretation, however, is lacking in the comparison of Phase 1 and Group TAP data. A more likely explanation of the greater homogeneity of Group TAR at testing lies in an

unidentified mechanism differentiating these animals from the parent generation and their TAP counterparts at conditioning.

In view of the proposition that Group TAR drinking was suppressed by indirect means, these animals may not be TA prone as comparison of Groups TAP and TAR consumption at testing would appear to indicate but rather, as breeding would predict, TA resistant. Examination of TAP and TAR difference scores, representing the change in fluid intake from conditioning to testing, provides little clarification. No divergence between the groups was observed; Groups TAP and TAR did not differ in the degree of post-conditioning drinking suppression. However, this finding refutes the supposition based on testing consumption data that the animals bred for resistance to developing an aversion (Group TAR) showed greater TA proneness than the group bred for enhanced TA learning efficiency (Group TAP).

The offspring generation may be further compared to the undifferentiated Phase 1 rats from which their parents were selected. No disparity in the difference scores (measures of drinking suppression) of either Group TAP or Group TAR as compared to Phase 1 animals was demonstrated, suggesting no observable influence of selective breeding for TA conditionability. In view of the contradictory support, previously noted, in lower testing consumption by Group TAP than the parent generation, the precise relevance of this

finding remains obscure. The results of the present experiment render insufficient evidence for any conclusion as to the presence or absence of a genetic predisposition to learning an aversion in the animals studied.

Regardless of the speculative interpretation forwarded, the fact remains that two distinct groups emerged. In maintaining these divergent lines in future assessments of their TA conditionability, enhancements to the experimental design are proposed.

First, in order to obtain more definitive results, alternative treatments should be considered. Rats have been shown to be highly sensitive to associating tastes with the US lithium chloride used in this experiment. Nachman and Ashe (1973) compared the effects of various volumes (1.0 to 20.0 ml/kg), concentrations (.15 to .65 M), and routes of administration (intraperitoneally, subcutaneously, and via stomach tube). Findings indicate the amount delivered rather than the concentration or route of administration is the determinant of a conditioned aversion. In the current study, the optimal dosage for producing an aversion, while subjecting the animals to minimal discomfort, was employed. Other methods of administration, such as distribution of two small doses of the drug as compared to the entire dosage in a single injection, have yielded more effective conditioning (Domjan, Foster, & Gillan, 1979). However, application of a more potent US risks creation of a floor effect, thereby

obscuring group differences in conditionability. The goal of observing potential group differences was best served by utilizing the threshold in treatment.

Options for assessing the effect of treatment, CS consumption, should be investigated. For example, a two-bottle preference test involving simultaneous delivery of the target solution and water has been endorsed in lieu of the single-bottle test (Dragoin, McCleary, & McCleary, 1971; Grote & Brown, 1971). Although the two-bottle test may be a more sensitive measure for detecting the occurrence of TA learning, this tool, because of its strength, may be inappropriate for discerning group differences. Batsell and Best (1993) present evidence supporting the technique of one-bottle testing as employed in the current study for distinguishing aversion strength between groups.

In addition, attention should be given to rigorous control of measurement error. Greater precision in consumption data was obtained through Phase 2 measures of mass, as opposed to volume recorded in Phase 1 and converted to mass for analysis. Finally, procedures and equipment which would effectively prevent fluid spillage observed during delivery and measurement are recommended.

The present experiment offers little clarification of the issues surrounding genetic contributions to the conditionability of an aversive response to taste. The results of this study remain ambiguous. Noteworthy, however,

are the initial findings of the prototype research first reported in 1983 (Elkins & Harrison, 1983) and reiterated in each update previously described. Progressive strain separation and significant strain differences in the intensity of conditioned taste aversion were demonstrated not in the first selected generation but beginning in the S-2 subjects. Continued development of the divergent lines of animals produced in this experiment may yet provide a replication of the results of the original program of research and contribute to the understanding of the basic phenomenon of taste aversion and its genetic components.

REFERENCES

Aronstam, R. S., Elkins, R. L., & Walters, P. A. (1990). Muscarinic acetylcholine receptor densities in brains of genetically selected taste-aversion-prone and resistant rats. Medical Science Research, 18, 213-214.

Bardo, M. T., & Valone, J. M. (1994). Morphine-conditioned analgesia using a taste cue: Dissociation of taste aversion and analgesia. Psychopharmacology, 114, 269-274.

Batsell, Jr., W. R., & Best, M. R. (1993). One bottle too many? Method of testing determines the detection of overshadowing and retention of taste aversions. Animal Learning and Behavior, 21, 154-158.

Bermudez-Rattoni, F., Forthman, D. L., Sanchez, M. A., Perez, J. L., & Garcia, J. (1988). Odor and taste aversions conditioned in anesthetized rats. Behavioral Neuroscience, 102, 726-732.

Best, M. R., & Barker, L. M. (1977). The nature of "learned safety" and its role in the delay of reinforcement gradient. In L. M. Barker, M. R. Best, & M. Domjan (Eds.), Learning mechanisms in food selection (pp. 295-317). Waco, TX: Baylor University Press.

Best, M. R., Batson, J. D., Meachum, C. L., Brown, E. R., & Ringer, M. (1985). Characteristics of taste-mediated environmental potentiation in rats. Learning and Motivation, 16, 190-209.

Best, P. J., Best, M. R., & Henggeler, S. (1977). The contribution of environmental non-ingestive cues in conditioning with aversive internal consequences. In L. M. Barker, M. R. Best, & M. Domjan (Eds.), Learning mechanisms in food selection (pp. 371-393). Waco, TX: Baylor University Press.

Bouton, M. E., Dunlap, C. M., & Swartzentruber, D. (1987). Potentiation of taste by another taste during compound aversion learning. Animal Learning and Behavior, 15, 433-438.

Bouton, M. E., & Whiting, M. R. (1982). Simultaneous odor-taste and taste-taste compounds in poison-avoidance learning. Learning and Motivation, 13, 472-494.

Buresova, O., & Bures, J. (1973). Cortical and subcortical components of the conditioned saccharin aversion. Physiology and Behavior, 11, 435-439.

Cannon, D. S., Best, M. R., Batson, J. D., Brown, E. R., Rubenstein, J. A., & Carrell, L. E. (1985). Interfering with taste aversion learning in rats: The role of associative interference. Appetite, 6, 1-19.

Cannon, D. S., & Carrell, L. E. (1987). Rat strain differences in ethanol self-administration and taste aversion learning. Pharmacology Biochemistry and Behavior, 28, 57-63.

Cannon, D. S., Leeka, J. K., & Block, A. K. (1994). Ethanol self-administration patterns and taste aversion learning across inbred rat strains. Pharmacology Biochemistry and Behavior, 47, 795-802.

Coburn, K. L., Garcia, J., Kiefer, S. W, & Rusiniak, K. W. (1984). Taste potentiation of poisoned odor by temporal contiguity. Behavioral Neuroscience, 98, 813-819.

Davis, S. F., Best, M. R., & Grover, C. A. (1988). Toxicosis-mediated potentiation in a taste/taste compound: Evidence for within-compound associations. Learning and Motivation, 19, 183-205.

Davis, S. F., Best, M. R., Grover, C. A., Bailey, S. A., Freeman, B. L., & Mayleben, M. A. (1990). The effects of taste extinction on ingestional potentiation in weanling rats. Animal Learning and Behavior, 18, 444-452.

De Beun, R., Rijk, H. W., & Broekkamp, C. L. E. (1993). Cross-familiarisation conditioned taste aversion procedure as a method to reveal stimulus resemblance between drugs: Studies on the 5-HT-sub(1A) agonist 8-OHDPAT. Psychopharmacology, 112, 121-128.

De Silva, P., & Rachman, S. (1987). Human food aversions: Nature and acquisition. Behavioural Research Therapy, 25, 457-468.

Domjan, M. Foster, K., & Gillan, D. J. (1979). Effects of distribution of the drug unconditioned stimulus on taste aversion learning. Psychology and Behavior, 23, 931-938.

Dragoin, W., McCleary, G. E., & McCleary, P. (1971). A comparison of two methods of measuring conditioned taste aversions. Behavioral Research Methods and Instrumentation, 3, 309-310.

Durlach, P. J., & Rescorla, R. A. (1980). Potentiation rather than overshadowing in flavor-aversion learning: An analysis in terms of within-compound associations. Journal of Experimental Psychology: Animal Behavior Processes, 6, 175-187.

Elkins, R. L. (1973). Individual differences in bait-shyness: Effects of drug dose and measurement technique. Psychological Record, 23, 349-358.

Elkins, R. L. (1974). Conditioned flavor aversions to familiar tap water in rats: An adjustment with implications for aversion therapy treatment of alcoholism and obesity. Journal of Abnormal Psychology, 83, 411-417.

Elkins, R. L. (1984). Taste-aversion retention: An animal experiment with implications for consummatory-aversion alcoholism treatments. Behavioural Research Therapy, 22, 179-186.

Elkins, R. L. (1986). Separation of taste-aversion-prone and taste-aversion-resistant rats through selective breeding: Implications for individual differences in conditionability and aversion-therapy alcoholism treatment. Behavioral Neuroscience, 100, 121-124.

Elkins, R. L. (1991). An appraisal of chemical aversion (emetic therapy) approaches to alcoholism treatment. Behavioural Research Therapy, 29, 387-413.

Elkins, R. L., Gerardot, R. J., & Hobbs, S. H. (1989). Differences in cyclophosphamide induced suppression of cricket predation in selectively bred strains of taste aversion prone and resistant rats. Behavioral Neuroscience, 103, 112-116.

Elkins, R. L., & Harrison, W. R. (1983). Rotation-induced taste aversion in strains of rats selectively bred for strong or weak acquisition of drug-induced taste aversions. Bulletin of the Psychonomic Society, 21, 57-60.

Elkins, R. L., & Walls, D. L. (1988). Ethanol-induced conditioned taste aversions and loss of righting reflexes in strains of selectively-bred taste-aversion-prone and resistant rats. Alcoholism: Clinical and Experimental Research, 12, 307.

Elkins, R. L., & Walters, P. A. (1990). Emetine induced taste aversions in rat strains selectively bred to differ in taste aversion conditioning. Alcoholism: Clinical and Experimental Research, 14, 285.

Elkins, R. L., Walters, P. A., Harrison, W. R., & Albrecht, W. (1990). Congruity of rotational and pharmacological taste aversion (TA) conditioning within strains of selectively bred TA prone and TA resistant rats. Learning and Motivation, 21, 190-198.

Elkins, R. L., Walters, P. A., & Orr, T. E. (1992). Continued development and unconditioned stimulus characterization of selectively bred lines of taste aversion prone and resistant rats. Alcoholism: Clinical and Experimental Research, 16, 928-934.

Elkins, R. L., Walters, P. A., Orr, T. E., Kolbe, E. F., Ritch, J. E., Hess, D. L., & Hobbs, S. H. (1991). Taste aversion, hypnotic and hypothermic effects of alcohol in rats genetically predisposed (selectively bred) to differ in taste aversion conditionability. Alcoholism: Clinical and Experimental Research, 15, 321.

Elkins, R. L., Walters, P. A., Orr, T. E., Kolbe, E. F., Westbrook, F., & Hobbs, S. H. (1991). Taste aversion inducing effects of cocaine in selectively-bred taste aversion prone and resistant rats. Neuroscience Abstracts, 17, 662.

Ellins, S. R., Cramer, R. E., & Whitmore, C. (1985). Taste potentiation of auditory aversions in rats (rattus norvegicus): A case for spatial contiguity. Journal of Comparative Psychology, 99, 108-111.

Estcorn, F., & Stephens, R. (1973). Establishment of conditioned taste aversions with a 24-hour CS-US interval. Physiological Psychology, 1, 251-253.

Galef, B. G. (1989). Socially mediated attenuation of taste-aversion learning in Norway rats: Preventing development of "food phobias." Animal Learning and Behavior, 17, 468-474.

Galef, Jr., B. G., & Osborne, B. (1978). Novel taste facilitation of the association of visual cues with toxicosis in rats. Journal of Comparative and Physiological Psychology, 92, 907-916.

Gamzu, E. (1977). The multi-faceted nature of taste-aversion-inducing agents: Is there a single common factor? In L. M. Barker, M. R. Best, & M. Domjan (Eds.), Learning mechanisms in food selection (pp. 477-509). Waco, TX: Baylor University Press.

Gamzu, E., Vincent, G., & Boff, E. (1985). A pharmacological perspective of drugs used in establishing conditioned food aversions. Annals of the New York Academy of Sciences, 443, 231-249.

Garb, J. L., & Stunkard, A. J. (1974). Taste aversions in man. American Journal of Psychiatry, 131, 1204-1207.

Garcia, J. (1989). Food for Tolman: Cognition and cathexis in concert. In T. Archer & L. Nilsson (Eds.), Aversion, avoidance, and anxiety: Perspectives on aversively motivated behavior (pp. 45-85). Hillsdale, NJ: Erlbaum.

Garcia, J., Ervin, F. R., & Koelling, R. A. (1966). Learning with prolonged delay of reinforcement. Psychonomic Science, 5, 121-122.

Garcia, J., & Koelling, R. A. (1966). Relation of cue to consequence in avoidance learning. Psychonomic Science, 4, 123-124.

Garcia, J., Lasiter, P. S., Bermudez-Rattoni, F., & Deems, D. A. (1985). A general theory of aversion learning. Annals of the New York Academy of Sciences, 443, 8-21.

Glass, G. V. (1970). Testing homogeneity of variances. In E. F. Heermann & L. A. Braskamp (Eds.), Readings in statistics for the behavioral sciences (pp. 323-327). Englewood Cliffs, NJ: Prentice-Hall.

Grote, F. W., & Brown, R. T. (1971). Conditioned taste aversions: Two-stimulus tests are more sensitive than one-stimulus tests. Behavioral Research Methods and Instrumentation, 3, 311-312.

Gustavson, C. R. (1977). Comparative and field aspects of learned food aversions. In L. M. Barker, M. R. Best, & M. Domjan (Eds.), Learning mechanisms in food selection (pp. 23-43). Waco, TX: Baylor University Press.

Hobbs, S. H., & Elkins, R. L. (1983). Operant performance of rats selectively bred for strong or weak acquisition of conditioned taste aversions. Bulletin of the Psychonomic Society, 21, 303-306.

Hobbs, S. H., Walters, III, P. A., Shealy, E. F., & Elkins, R. L. (1993). Radial-maze learning by lines of taste-aversion-prone and taste-aversion-resistant rats. Bulletin of the Psychonomic Society, 31, 171-174.

Kalat, J. W. (1977). Status of "learned safety" or "learned noncorrelation" as a mechanism in taste-aversion learning. In L. M. Barker, M. R. Best, & M. Domjan (Eds.), Learning mechanisms in food selection (pp. 273-293). Waco, TX: Baylor University Press.

Kalat, J. W., & Rozin, P. (1971). Role of interference in taste-aversion learning. Journal of Comparative and Physiological Psychology, 77, 53-58.

Kalat, J. W., & Rozin, P. (1973). "Learned safety" as a mechanism in long-delay taste-aversion learning in rats. Journal of Comparative and Physiological Psychology, 83, 198-207.

Kimble, G. A. (1961). Hilgard and Marquis' conditioning and learning (2nd ed.). New York: Appleton.

Kirk, R. E. (1968). Experimental design: Procedures for the behavioral sciences. Belmont, CA: Brooks/Cole.

Logue, A. W. (1985). Conditioned food aversion learning in humans. Annals of the New York Academy of Sciences, 443, 316-329.

Lubow, R. E. (1973). Latent inhibition. Psychological Bulletin, 79, 398-407.

Mark, G. P., Blander, D. S., & Hoebel, B. G. (1991). A conditioned stimulus decreases extracellular dopamine in the nucleus accumbens after the development of a learned taste aversion. Brain Research, 551, 308-310.

Nachman, M., & Ashe, J. H. (1973). Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. Physiology and Behavior, 10, 73-78.

Orr, T. E., Walters, P. A., Carl, G. F., & Elkins, R. L. (1993). Brain levels of amines and amino acids in taste aversion-prone and -resistant rats. Physiology & Behavior, 53, 495-500.

Pelchat, M. L., & Rozin, P. (1982). The special role of nausea in the acquisition of food dislikes by humans. Appetite, 3, 341-351.

Rescorla, R. A. (1981). Simultaneous associations. In P. Harzem & M. D. Zeiler (Eds.), Advances in analysis of behaviour: Vol. 2. Predictability, correlation, and contiguity (pp. 47-80). New York: Wiley.

Revusky, S. (1971). The role of interference in association over a delay. In W. K. Honig, & P. H. R. James (Eds.), Animal memory (pp. 155-213). New York: Academic Press.

Riley, A. L., Jacobs, Jr., W. J., & Mastropaolo, J. P. (1983). The effects of extensive taste preexposure on the acquisition of conditioned taste aversions. Bulletin of the Psychonomic Society, 21, 221-224.

Riley, A. L., & Tuck, D. L. (1985). Conditioned food aversions: A bibliography. Annals of the New York Academy of Sciences, 443, 381-437.

Roll, D. L., & Smith, J. C. (1972). Conditioned taste aversion in anesthetized rats. In M. E. P. Seligman & J. L. Hager (Eds.), Biological boundaries of learning (pp. 98-102). New York: Appleton-Century-Crofts.

Rozin, P. (1976). The evolution of intelligence and access to the cognitive unconscious. In J. A. Sprague & A. N. Epstein (Eds.), Progress in psychobiology and physiological psychology (Vol. 6, pp. 245-280). New York: Academic Press.

Rozin, P. (1977). The significance of learning mechanisms in food selection: Some biology, psychology and sociology of science. In L. M. Barker, M. R. Best, & M. Domjan (Eds.), Learning mechanisms in food selection (pp. 557-589). Waco, TX: Baylor University Press.

Rozin, P. (1986). One-trial acquired likes and dislikes in humans: Disgust as a US, food predominance, and negative learning predominance. Learning and Motivation, 17, 180-189.

Rozin, P., & Kalat, J. W. (1971). Specific hungers and poison avoidance as adaptive specializations of learning. Psychological Review, 78, 459-486.

Seligman, M. E. P. (1970). On the generality of the laws of learning. Psychological Review, 77, 406-418.

Smith, J. C., & Roll, D. L. (1967). Trace conditioning with x-rays as an aversive stimulus. Psychonomic Science, 9, 11-12.

Zahorik, D. M., & Houpt, K. A. (1977). The concept of nutritional wisdom: Applicability of laboratory learning models to large herbivores. In L. M. Barker, M. R. Best, & M. Domjan (Eds.), Learning mechanisms in food selection (pp. 45-67). Waco, TX: Baylor University Press.

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