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Charles N. Stewart,[1] *Russell F. Reidinger, Jr.,*[2] *and*
J. Russell Mason[3]

A Method for Inferring the Taste Qualities of Rodenticides to Rodents

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ABSTRACT: A method recently developed for the study of animal psychophysics was adapted for use in inferring the taste characteristics of rodenticides, as perceived by rodents. The method is based on generalization of learned food aversions to other materials having similar tastes. The potential of the method was illustrated in two experiments. In Experiment 1, five specimens of *Rattus norvegicus* (Sprague-Dawley) were conditioned to avoid the taste of strychnine (alkaloid) by pairing brief exposures to the strychnine with intraperitoneal injections of lithium chloride (LiCl). In subsequent 15-min drinking tests, the rats avoided mainly quinine sulfate and sucrose octaacetate [but not sodium chloride (NaCl) or hydrochloric acid (HCl), and sucrose only slightly] when compared with control rats. These results were confirmed in Experiment 2, and results from this experiment also indicated that sodium bicarbonate was not an effective agent for masking the taste of strychnine (that is, four rats conditioned with strychnine containing sodium bicarbonate still avoided quinine sulfate, compared with control rats in subsequent drinking tests, and four rats conditioned with only strychnine avoided quinine sulfate containing sodium bicarbonate in subsequent drinking tests). We suggest that the method has potential for use in assessing taste characteristics of rodenticides, as well as such bait additives as taste enhancers and masking agents, to rodents.

KEY WORDS: food aversion learning, rodenticide, sodium bicarbonate, strychnine, taste, taste masking, vertebrate pest control

Various approaches are used to gather information on the taste qualities (sweet, sour, salty, bitter) of materials to nonhuman species. All of the ap-

[1]Professor of psychology, Whitely Psychology Laboratories, Franklin and Marshall College, Lancaster, Pa. 17604.

[2]Wildlife biologist, U.S. Fish and Wildlife Service, assigned to the Monell Chemical Senses Center, Philadelphia, Pa. 19104, from the Denver Wildlife Research Center, Section of Supporting Sciences, Lakewood, Colo. 80225

[3]Assistant member, Monell Chemical Senses Center, Philadelphia, Pa. 19104.

proaches are necessarily indirect [*1*], and each has its own advantages and disadvantages. One approach, the simplest, is to base descriptions of taste qualities on the psychophysical responses of humans to the materials and to assume that human responses are similar to those of other animals. Unfortunately, the perceived taste of a material to humans may not reflect the perceived taste qualities of the same material to other animals. For example, cyclamate tastes sweet to humans at the same concentration that it is believed to taste bitter to rats and salty to hamsters [*2*].

In a second approach, an electrophysiological one, the animal is placed under anesthesia, and the chorda tympani or glossopharyngeal nerves, or single nerve fibers, are connected to microelectrodes in such a manner that summated single and multiunit electrical activity can be monitored. Differential neuronal activity in response to applications of tastants [such as sodium chloride (NaCl), sucrose, quinine hydrochloride, and hydrochloric acid (HCl)] to the tongue is then recorded. The method has been used to infer taste qualities of chemicals and to provide information on detection thresholds and identification thresholds of the compounds. Although used successfully in many studies of animal psychophysics [*1*], the method is limited by the complexity of experimental preparation, the time required to achieve large sample sizes, differential responses of the chorda tympani and the glossopharyngeal nerves to different taste qualities [*3*], and uncertainties regarding the relationships between attributes of neuronal activity (such as amplitude and frequency) and perception of taste qualities [*3*].

A third approach is based on work by Nachman and others. Nachman [*4*] and Nachman et al [*5*] presented lithium chloride (LiCl) in drinking water to rats who subsequently formed learned aversions specific to the taste of LiCl. Following this conditioning, the workers demonstrated that avoidance generalized to other compounds depending on the similarity of their tastes to that of LiCl. Thus, NaCl, which closely resembles LiCl in structure and probably in taste, was strongly avoided. Compounds such as potassium chloride (KCl) and ammonium bicarbonate (NH_4CO_3) were less strongly avoided. Nowlis and Frank [*6*] and Nowlis et al [*2*] have also used generalization of learned food aversions to describe taste qualities in rat and hamster taste perception. Animals were exposed to a taste [that is, a conditioned stimulus (CS)] and then sickened by injections of apomorphine hydrochloride [the unconditioned stimulus (US)]. In subsequent tests, Nowlis and co-workers recorded generalization of the learned aversions from the conditioned stimuli to a variety of compounds (test stimuli). One advantage of this approach is that it allowed the collection of taste information on large numbers of animals in a relatively short time. A limitation is that responses of the animals to the test stimuli may be confounded by differential hedonic responses (that is, unlearned likes and dislikes) to different taste qualities.

A fourth approach is to train the animal to indicate its discrimination between two taste qualities by some behavioral response, such as bar pressing or licking, in order to receive a food or water reward. The approach provides a motivation for behavioral response other than taste quality and has been used by Morrison [*7*] to assess the taste qualities of chemicals to rats. The approach has the additional advantage of not requiring that the animal be made sick in order to display taste discrimination but the disadvantage of again requiring long periods of time to train and test large numbers of rats.

A better understanding of how rodenticides taste to rodents could lead to improvements in rodent control (see Ref *8*). Two of the methods previously described have already been used to gather limited information on this issue. Richter [*9*] used preference tests to determine detection thresholds (that is, the minimum concentrations at which rodenticides could be detected) of seven rodenticides [alpha-naphthylthiourea (ANTU), arsenic trioxide, mercuric chloride, phenylthiourea, red squill, strychnine sulfate, and thiosemicarbazide] to *Rattus norvegicus*. He also requested a panel of 60 adult human volunteers to judge the bitterness of the compounds (the first approach). Kusano and colleagues have used the electrophysiological approach (the second approach described earlier), sometimes in conjunction with behavioral tests, to infer the taste qualities of thiourea derivatives (including ANTU) [*10,11*], thallous nitrate [*12,13*], and cycloheximide [*14*] to *R. norvegicus*.

We report here findings from an initial assessment of the use of generalization of learned aversions, the third approach, as a method to infer the taste qualities of rodenticides as perceived by rats. In Experiment 1, we used the method to describe the taste properties of strychnine to *R. norvegicus*, laboratory strain. In Experiment 2, we used the method to assess the effectiveness of sodium bicarbonate as a masking agent for the taste of strychnine. Strychnine was selected because Richter [*9*] had already provided information on the detection thresholds of rats to the compound. Sodium bicarbonate has been reported as a masking agent for the taste of the compound, but its function in this capacity has been questioned since Ward and Munch (using the first approach) [*15*] reported that sodium bicarbonate enhanced, rather than masked, the taste of strychnine in humans. We were particularly interested in the sensitivity of the method with regard to bitter tastes, in part because the method relies on consumption during drinking tests, and bitter-tasting materials are known to be relatively unpalatable to rats, and in part because many rodenticides, particularly the acute ones (including strychnine), are believed to have bitter taste components. Our choices of subjects and compounds were therefore logistically advantageous for an initial evaluation of the method but precluded immediate practical applications of the findings.

Materials and Methods

The subjects were adult male rats (*R. norvegicus*, Sprague-Dawley strain[4]), individually housed with free access to Wayne Lab Blox and to tap water, be-

[4]Perfection Breeders, Inc., Douglassville, Pa. References to trade names do not imply U.S. government endorsement of commercial products.

fore the experiments. The colony was maintained at about 22°C with a 12 h: 12 h light:dark cycle. For Experiment 1, we used 10 adult male rats weighing 450 to 550 g; for Experiment 2, we used 24 adult male rats weighing 396 to 473 g.

Chemical Stimuli

The following compounds, in distilled water, were used as conditioned stimuli: for both experiments, strychnine (levorotatory alkaloid—Aldrich, F. W. 334.42) at 0.0002*M*; for Experiment 2, a mixture of strychnine at 0.0002*M* containing sodium bicarbonate at 0.0016*M* and sodium bicarbonate at 0.0016*M*. In both experiments, LiCl (0.15*M*) injected intraperitoneally at 102 mg/kg body weight served as an unconditioned stimulus. In Experiment 2, NaCl (0.15*M*) injected intraperitoneally at 140 mg/kg was also used as the unconditioned stimulus for control rats. The following compounds, in distilled water, were used as test stimuli for both experiments: 0.1*M* sucrose (*S*), 0.1*M* NaCl (*N*), 0.01*M* HCl (*H*), and 0.0001*M* quinine sulfate (*Q*). In addition, 0.001*M* sucrose octaacetate (*O*) served as a test stimulus in Experiment 1, and 0.0001*M* quinine sulfate containing 0.0019*M* sodium bicarbonate served as an additional test stimulus (*BQ*) in Experiment 2. Sodium bicarbonate was added to the strychnine in the ratio of 2:1 (by weight) as reportedly used in bait formulations for control of ground squirrels.[5] The concentrations of the test stimuli were similar to those previously shown to be useful in generalization tests with other substances and rodents [*2,6*]. The strychnine concentration was selected so as to be equimolar to the highest detection threshold value reported by Richter [*9*], and thus one which would probably be tasted by all of the rats.

Adaptation

Before each experiment, the animals were adapted for at least seven days to a schedule of fluid deprivation, following the procedure of Nowlis et al [*2*]. The schedule was followed throughout the experiments. Each rat was given access to distilled water for 15 min (test period) during the first 6 h of the light cycle and to tap water for 30 min (hydration period) during the last 6 h of the light cycle. Fluids were presented in Richter tubes, calibrated in millilitres, and attached individually to the fronts of the cages.

Procedure

Experiment 1—For the last five days of adaptation, the rats were rank ordered according to their mean drinking scores during the test periods. Of those two animals with the lowest drinking scores, one was assigned randomly

[5]J. Bean, manager, Pocatello Supply Depot, Pocatello, Idaho 83201, personal communication, 1981.

to an experimental group and the other was assigned to a control group. Of those two animals with the next lowest drinking scores, one was assigned at random to the experimental group and the other to the control group, and so forth, so that the experimental group ($n = 5$) and the control group ($n = 5$) were balanced according to average fluid intakes.

On the day of LiCl dosing, the rats in the experimental group were allowed to drink the strychnine solution for 5 min during the test period, whereas the rats in the control group were presented with only the distilled water. Five minutes after drinking, each rat received an injection of LiCl. For two days following conditioning, the rats were given only tap water (*ad libitum*) to allow them to recover from the immediate effects of LiCl (or other aspects of the procedure) before testing the rats' responses to the solutions of tastants.

During the 15-min test period for five days thereafter, each rat was presented with 30 mL of a test stimulus (*H, N, O, Q*, or *S*), and consumption was recorded at the end of the period. One-bottle drinking tests were used instead of two-bottle tests, even though the former are less sensitive for detecting taste aversions [*16*], to encourage measurable consumption of *O* and *S*. Presentations of each of the five test stimuli were repeated for two additional weeks (following a Latin square design) so that all the rats had been exposed to each test stimulus three times over the course of three weeks.

Experiment 2—The same general procedure was used for Experiment 2. In this experiment, however, the last two days of adaptation (rather than the last five days) were used to rank order the rats, and the rats were assigned to six groups ($n = 4$ per group). Three of the groups (1 through 3) were experimental, and the others were control groups matched to experimental groups on the basis of drinking scores. On the day of conditioning, the rats were allowed to drink the following conditioned stimuli for 5 min during the test period: Group 1 and its control group, strychnine solution; Group 2 and its control group, strychnine solution containing sodium bicarbonate; and Group 3 and its control group, sodium bicarbonate solution. Five minutes after drinking, the rats in Groups 1 through 3 received injections of LiCl whereas the rats in the control groups received injections of NaCl (isotonic, 0.9% weight/volume). As in Experiment 1, the rats were then allowed two days to recover from the effects of LiCl. During the 15-min test period for five days thereafter, each rat was presented with 30 mL of a test stimulus (*BQ, H, N, Q*, or *S*) in one-bottle tests, and consumption was recorded at the end of the period. Taste presentations were repeated again for a second week (following a Latin square design) so that all the rats were exposed to each test stimulus twice.

Analysis

The data on consumption were converted to suppression ratios (defined as the amount of a test stimulus consumed by each experimental animal divided by the amount consumed by its control, the quotient subtracted from unity and multiplied by 100), following the procedure of Nowlis et al [2] and Nowlis and Frank [6]. Although statistical analyses of suppression ratios are possible [2], mean suppression ratios of groups served adequately here as the bases for our descriptions of taste characteristics of the conditioned stimuli as perceived by *R. norvegicus*.

Results and Discussion

Experiment 1

Following association of the taste of strychnine with LiCl-induced sickness, the experimental rats suppressed their consumption of (that is, generalized their aversions to) *Q* and *O* compared to the consumption of these solutions by control rats (Fig. 1, Week 1). Avoidance of *Q* persisted during both additional exposures in Weeks 2 and 3 of the tests, but avoidance of *O* attenuated by the second exposure on Week 2. We concluded that aversions to strychnine generalized primarily to those substances that humans describe as bitter. The relatively rapid attenuation of avoidance to *O* could be explained as reflecting different strengths of taste of the two solutions or as representing two different types of tastes in the rat taste system, with *Q* more closely resembling the taste of strychnine than *O*.

Experiment 2

Following association of the taste of strychnine with LiCl-induced illness, Group 1 rats strongly avoided *Q* (Fig. 2). The percent suppression of *Q* intake was less in Experiment 2 than in Experiment 1, a finding which could be attributed to a change in the procedure in which the control group was given exposure to the strychnine unpaired with LiCl rather than to LiCl alone. The postingestional effects of the strychnine alone may have produced a weak aversion to that taste in the controls, thus lessening differences between the experimental animals and the controls. Because the rats in Group 2 avoided both *Q* and *BQ*, but not those in Group 3 who had been conditioned with only sodium bicarbonate as a CS, we suggest that sodium bicarbonate was ineffective in masking the taste of quinine. This notion is further supported in that Group 2 rats, conditioned with a mixture of strychnine and sodium bicarbonate, also suppressed their drinking of both *Q* and *BQ* to an extent similar to that demonstrated by the rats in Group 1 (Fig. 2). These findings are consistent with those of Ward and Munch [*15*] that sodium bicarbonate was ineffective in masking the bitter taste of strychnine to humans. Group 2 rats also avoided *N*, perhaps in response to the sodium in the sodium bicarbonate portion of their CS. Such a response was not observed in Group 3 rats, perhaps because the Na^+ taste was not potentiated by an association with strychnine (as it may have been for the Group 2 rats).

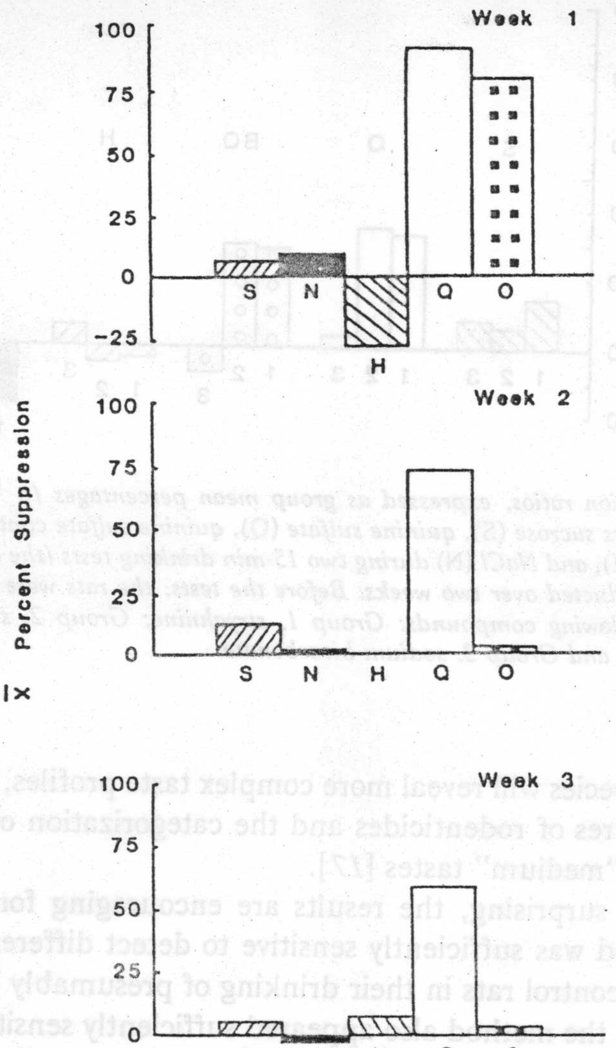

FIG. 1—*Suppression ratios, expressed as mean percentages (*n = *5), exhibited to the tastants sucrose* (S), *HCl* (H), *NaCl* (N), *quinine sulfate* (Q), *and sucrose octaacetate* (O) *during three 15-min drinking tests conducted over three weeks. Before the tests, the rats were conditioned to avoid the taste of strychnine.*

General Discussion

To our knowledge, the results represent the first assessment of the taste characteristics of a rodenticide using generalization of learned aversions. The finding that the taste of strychnine generalized primarily to *Q* and *O*, and that by inference the taste of strychnine appeared to be primarily bitter to the rats, is not surprising. These results are consistent with both Richter's findings [*9*], from his study involving a human taste panel, and the common categorization of strychnine (for example, Ref *17*) as having a "strong" taste, "normally objectionable to rats." The results are also consistent with the more general notion (for example, Ref *18*) that bitter tastes connote natural poison and are avoided by rats. We suspect, however, that studies with some other rodenti-

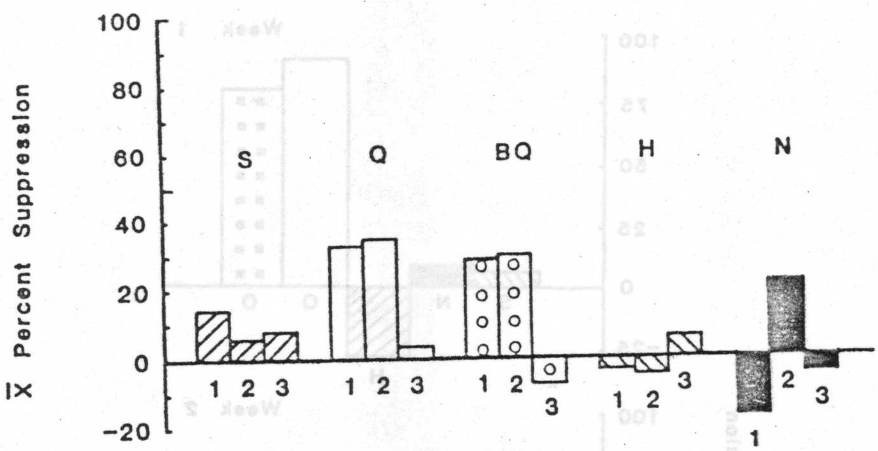

FIG. 2—*Suppression ratios, expressed as group mean percentages (n = 4 per group), exhibited to the tastants sucrose* (S), *quinine sulfate* (Q), *quinine sulfate containing sodium bicarbonate* (BQ), *HCl* (H), *and NaCl* (N) *during two 15-min drinking tests (the data for the two tests were combined) conducted over two weeks. Before the tests, the rats were conditioned to avoid the tastes of the following compounds: Group 1, strychnine; Group 2, strychnine containing sodium bicarbonate; and Group 3, sodium bicarbonate.*

cides and pest species will reveal more complex taste profiles, given the diverse chemical structures of rodenticides and the categorization of some as having only "slight" or "medium" tastes [*17*].

Although not surprising, the results are encouraging for several reasons. First, the method was sufficiently sensitive to detect differences between experimental and control rats in their drinking of presumably bitter-tasting test stimuli. Second, the method also appeared sufficiently sensitive to detect possibly subtle nuances in the taste of strychnine; that is, it tasted more like *Q* than *O* (at the concentrations used). Third, the method also provided support for the observation that sodium bicarbonate does not act as a masking agent for strychnine. Additional tests would be required, of course, to determine if pest rats respond in a manner similar to that of albino laboratory rats. However, some available evidence suggests that they would [*19*].

Obviously, the method requires additional refinement and evaluation before its full potential for use in the practical improvements of rodenticides and bait formulations is known. In interpreting our results from this study, we used the term "taste characteristics" loosely and cannot preclude the possibility that characteristics other than taste quality, such as texture, related odors, viscosity, or pH, served as conditioned stimuli for the learned aversions. Basic work is needed on the possible mediating role of such variables.

We have also explored the use of two different control group procedures, one (Experiment 1) in which the animals were given the illness-inducing agent (LiCl) alone (unconditioned stimulus), and the other (Experiment 2) in which animals were given a taste of the rodenticide (conditioned stimulus) alone. Both procedures were consistent and thus effective in comparison with the experimental groups, but we would recommend the use of the LiCl-alone control because it avoids the possibility of control animals forming an aversion to the taste of the rodenticide by virtue of its postingestional consequences.

Although we presented the rodenticide-CS in drinking water, we believe that the procedure could be modified to work with solid rodenticides and baits. For example, rodents could be presented with small amounts of a solid bait containing or not containing a rodenticide, then injected with LiCl and tested for generalization to liquid test stimuli, thus enabling one to determine the influence of the added rodenticide on the taste characteristics of the bait. By using analogous procedures, one might be able to test the effectiveness of various microencapsulating techniques or masking agents. By increasing the number of test stimuli, and by employing such procedures as multidimensional scaling in analyzing the suppression ratios [*6*], more refined descriptions of the taste qualities of rodenticides might be obtained. Such descriptions might be particularly useful in the development and assessment of complex bait formulations and in the improvement of prebaits through the addition of taste simulants, as suggested by Robbins [*20*].

In summary, the method provided results that were consistent with other studies on, and knowledge of, the taste properties of strychnine and sodium bicarbonate. The method appears to have potential for practical contributions to improvements in rodenticides and bait formulations.

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