METHODOLOGY FOR MEASURING TASTE AND ODOR PREFERENCE OF RODENTS[1]

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ABSTRACT: Taste enhancers and olfactory attractants are needed to improve bait acceptance for rodent control, but most methods for evaluating preference for taste and odor stimuli are not suitable for screening large numbers of such compounds. This paper describes two automated preference testers designed for this purpose. The taste preference apparatus is based on the principle of the brief-exposure, foods-together technique, whereby the animal briefly samples each food alone, in alternate sequence, before the two foods are presented together, in alternate positions. The odor preference tester is based on an open-field maze, whereby the test animal samples each of four odor sources before preference behavior is recorded. Both devices are fully automated (in both operation and data recording), are free of position bias, and produce preference determinations in relatively little time; neither requires special training of test animals. The design, operation, and application of each apparatus in rodent control is discussed and illustrated.

INTRODUCTION

There is a need in the field of rodent damage control for taste enhancers and olfactory attractants to permit formulation of highly palatable bait carriers with attractant properties. To achieve this objective, laboratories need a fast and sensitive method of evaluating candidate compounds. However, test results obtained from screening such compounds are often unpredictable. Results depend on rodents' preference behavior, which is complex and influenced by many factors such as the animal's previous experience, sex, age, deprivation level, cues learned in testing, positional bias, postingestinal effects, concentration of the chemical, contamination with other stimuli, etc. Most test designs that are economical and simple produce results that are not sensitive because animals are not given a choice or because test results are based on 24-hour consumption data, which confounds initial preference with postingestinal effects (Young 1967; Shumake et al. 1971). In two-choice, 24-hour consumption tests, an animal may choose a food because of its location rather than its palatability. Similarly, a forced choice situation can produce erroneous results. As an example, Young and Green (1953) have shown that rats ingest more of a 9-percent sucrose than a 36-percent sucrose solution when each solution is presented alone. However, if the two solutions are presented together, rats will ingest more of the 36-percent solution.

When we began our program to develop taste and odor methodology, we began to search the literature for baseline data on taste and olfaction in rodents and for testing techniques that would overcome some of the confounding factors of commonly used bioassay techniques. In reviewing the literature, we found that, while there is a large volume of literature (Pangborn and Trabue 1967; Cheal and Sprott 1971) on taste and olfaction of domesticated laboratory rats, much of it appears to be of little apparent value in rodent control. In addition, most of the reports on taste preference pertain to liquids representing the four basic taste qualities (sweet, sour, bitter, and salty). Of the few tests with solid foods, most have been cage bioassays where preference is based on 24-hour consumption; this, as has been mentioned, is not a very sensitive test method.

There has been even less work on the attractant properties of odors to rodents, even though it seems clear that the odor of bait materials has an important influence on approach behavior (Reif 1956; Howard and Cole 1967; Howard et al. 1968, 1969; Howard and Marsh 1970). This situation apparently stems from a past emphasis on studying odor perception and discrimination of laboratory rats and a lack of standardized methodology for measuring attractancy. Of the several odor-testing devices reported in the literature, some are based on

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operant conditioning techniques (Pfaffman et al. 1958; Eayrs and Moulton 1960; Goff 1961) and are not applicable to a laboratory screening operation. Howard et al. (1969) described a body capacitor-olfactometer chamber that seems to give reliable results, but it does not appear to be applicable to large-scale screening because of the length of time required to conduct tests and the difficulty of eliminating odor contamination between tests. Long and Tapp (1968) described a lever-pressing apparatus for assessing the reinforcing properties of odors; although the principle looks promising, unpublished test results obtained with the device at this laboratory (Thompson et al. 1969) and at Stanford Research Institute (Pryor and Otis 1970) have been unsatisfactory.

To obtain a test system that was simple and sensitive enough for screening, it was apparent that we would have to design our own apparatus. The system originally introduced by Young and Kappauf (1962) for measuring taste preference of rats for liquids seemed the best place to start. Their design was based on the brief-exposure, foods-together technique, which eliminated many of the potential biases of other systems. By this technique, the test animal is given a two-choice situation; however, the animal briefly samples each food alone in alternate sequence before the two foods are presented together, in alternate positions. Alternating the sequence and positions in which foods are presented minimizes both temporal and positional habits. According to Young (1967), brief-exposure preference tests are best because the influence of acquired habits is effectively removed and tests are completed before postingestinal factors influence the result. In this technique, the choice is the important parameter, and the large number of choices is a statistical asset.

We used this principle as a basis for building a semi-automatic preference tester for taste stimuli. Later we built an odor preference tester based on a modification of this concept. We have found these two devices both useful and sensitive in screening tastes and odors, and we will briefly describe them here.

DESCRIPTION

Taste Preference Tester

This apparatus, which can use either a liquid or solid food base, is described in detail by Thompson and Grant (1971). Briefly, it consists of a six-compartment circular food tray, two photobeams with receivers, a reversible motor, a gear drive system, and a limit switch to control positioning of the food tray. These components are enclosed in a 17.2 x 14.0 x 17.6 cm Plexiglas[1] box (module 1) and are connected by a multiconductor cable to a remote master control-recording module (module 2). The front panel of module 1 has a stainless steel covering and a 5.1 x 7.0 cm food port. It is placed in the front of the test animal's holding cage when a preference determination is to be made. When the animal eats from the food compartments, the photobeam is interrupted; the resulting voltage change is amplified and closes a recording relay in module 2.

There are four food tray positions: two "alone" (A and B) and two "choice" (AB and BA). Before a preference determination is made, animals are trained to eat from the tray when all compartments contain the same food. To determine preference, one food is placed in the three A compartments and an equal amount of another food is placed in the three B compartments; one food serves as a "standard" and the other as a "test" food. Module 1 is then placed in the animal's cage in either of the "alone" positions, along with drinking water. The tester is programmed so that as soon as the animal has eaten from one of the food tray compartments for an accumulated preset time, the tray automatically rotates to another of the four positions. In a typical choice cycle, the animal samples food A, samples food B, chooses between A and B presented simultaneously, samples food B, samples food A, chooses between B and A (positions reversed). This sequence is repeated until the animal makes enough choices to determine preference. The time spent eating foods A and B in the choice positions, the number of times the standard food and the test food are chosen, and the number of food-choice presentations are summed by digital counters in module 2. At the end of the testing period, module 1 is removed from the cage, and the food remaining in each compartment is weighed. Preference ratings (P) are computed for each animal by the formula $P = 100T/(T+S)$, where T is the weight of the test food consumed (or time spent eating the test food) and S is the weight of the standard food eaten (or time spent eating the standard food).

[1]Reference to trade names does not imply endorsement of commercial products by the Federal Government.

In preliminary uniformity tests with 38 black hooded rats and all compartments containing the same food, preference for the A and B compartments was almost exactly 50:50, indicating no positional bias (Thompson and Grant 1971). Some of the possibilities for practical preference testing with the apparatus were demonstrated in an experiment comparing the taste responses of wild Norway rats (Rattus norvegicus) and laboratory rats (Shumake et al. 1971). Test results from this experiment are reproduced in Figure 1.

Odor Preference Tester

This open-field odor-testing device is currently being described in detail for publication[1]. The entire device is constructed with chemically inert materials (Teflon, Plexiglas, glass, and stainless steel) that are easily cleaned and relatively odor-free. Briefly, it consists of a circular open-field area 2 ft in diameter and four 2-ft-long glass odor-emission tubes connected at right angles to it. A Plexiglas cover is suspended on rollers over the open field area. Attached to the periphery of the cover are four gates made of stainless steel rods to block the entrances of the odor tubes. The center of the cover contains a small exhaust fan that slowly draws equal air currents through each tube. A single rat is introduced through a hinged cover under the exhaust fan. As it explores the periphery of the open-field area, it makes nose and mouth contact with the stainless steel gates, each of which is connected to a "drink-o-meter" circuit that detects contact. After the rat has made contact with all gates, regardless of sequence, a small amount of 0.5 percent sucrose solution is automatically injected into a drinking fount near the center of the open field area. When the rat eventually returns to the center of the field and drinks the sucrose solution, a fifth drink-o-meter circuit starts a small reversible motor that drives the circular cover 21 degrees, removing the gates from all four odor tubes. A free four-choice condition is then in effect. Photocells positioned in front of each odor source detect both the number of times each odor is visited and the time spent in the presence of each odor; these data are recorded on a remote digital counter. After each rat has been tested and removed, the motor reverses to close the gates and the entire device cleans itself with two hot water sprayers.

The odor preference tester has been used in experiments with laboratory rats, wild Norway rats, and ricefield rats (Rattus rattus mindanensis). In a uniformity test with 20 domesticated Norway rats and the same food odor in all four tubes, no statistically significant difference was detected between the four tubes in either number of visits or elapsed time. In preliminary tests to determine if 20 domesticated Norway rats could locate a urine or food odor when the other three tubes were odorless controls (deionized water), significant preferences ($P<0.01$) were shown, in both number of visits and elapsed time, for the tube emitting the odor. Thus it appears that there is no positional bias in the apparatus and that test rats can locate and respond to preferred odors.

Table 1 shows an example of the kind of results that can be obtained with the odor tester. Twenty candidate attractants were compared with a food odor standard (Purina Laboratory Chow) and a water control and ranked for attractancy by visitation frequency (number of photocell interruptions in a 30-minute test session). The lowest frequency was assigned a rank of 1 and the highest, 4; equal observations were assigned mean ranks. The percent of food odor response (P) for each compound and test animal was then computed by the formula $P = 100RC/RF$, where RC is the rank of the candidate attractant and RF is the rank of the standard food odor. (In this system, $P = 100$ indicates that the candidate compound is equal in preference to the standard food odor.) Preference ratings for each compound were averaged for each group of animals and arrayed. It is interesting to note that all the compounds ranked below the standard food odor, indicating that none of them are especially strong rodent attractants.

DISCUSSION

The taste preference device offers many refinements over the two-choice, 12- or 24-hour cage test commonly used for large-scale screening. Module 1 weighs only 5-1/2 lb and is thus easily moved from cage to cage. The device's noise-producing components (module 2) are isolated from test animals in a partially sound-proofed adjoining room. No physical handling of the animals is necessary, since the test apparatus is placed directly into the home cage; this minimizes stress and tends to reduce orientation time. The automated food

[1] Shumake, S.A., R.D. Thompson, and R.W. Bullard. An automated open-field odor test maze for rats (in ms.).

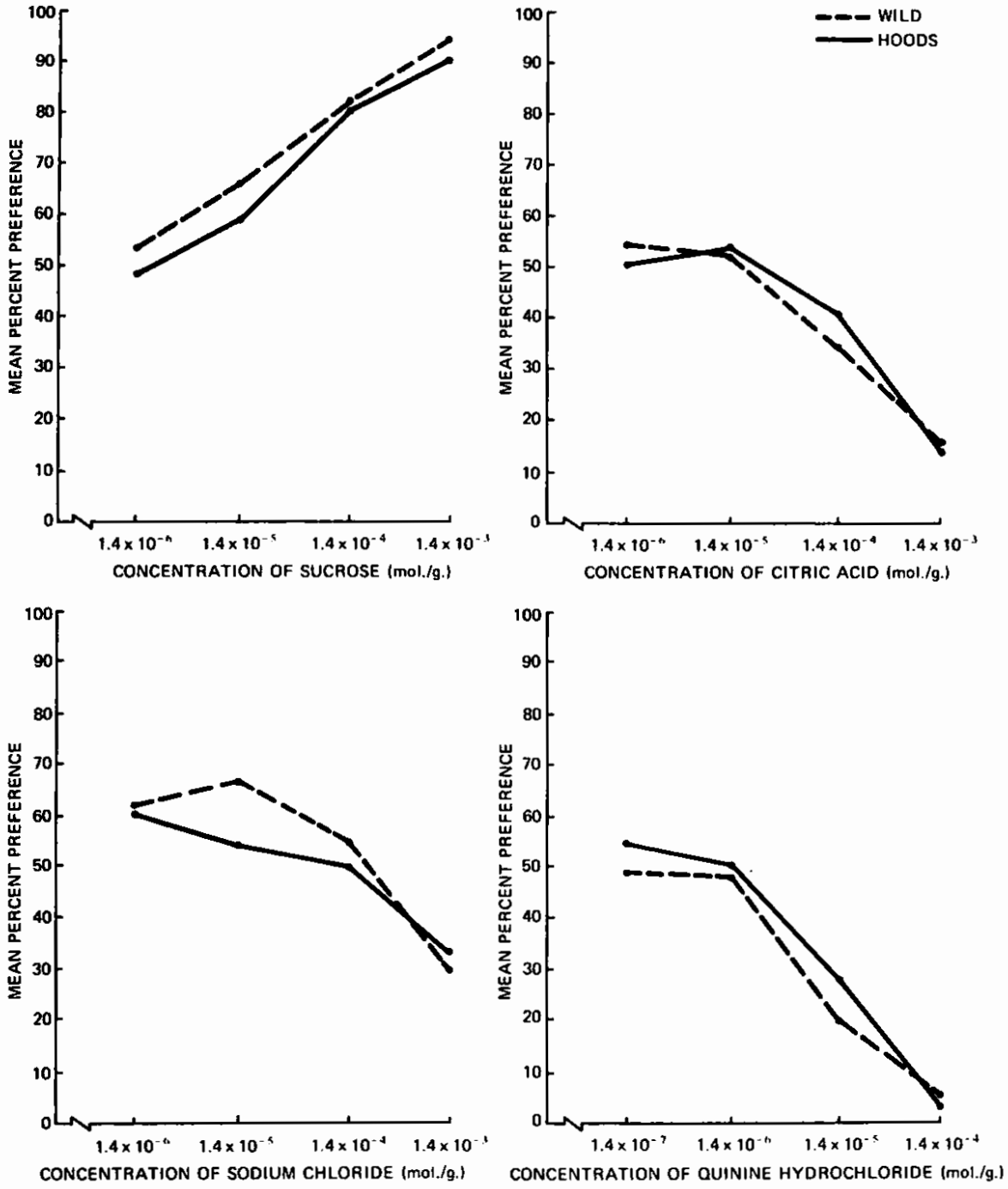

Fig. 1. Mean percentage preference response of hooded and wild Norway rats to four concentrations of four taste stimuli. (From Shumake, Thompson, and Caudill. 1971. J. Comp. Physiol. Psychol. 77:492. Copyright by the American Psychological Association, and reproduced by permission.)

presentation, under control by the animal, makes explicit training unnecessary. Two to five, 30-minute orientation periods are required for laboratory rats to adapt to the movement and turning sounds of the motor. Wild rats usually require longer periods of exposure and moderate food deprivation. Oriented animals usually make 25, 6-second choices in a 30-minute test period, which, according to Young and Madsen (1963), is an adequate number of choices for taste preference determination.

Table 1. Results with odor preference tester. Attractancy ranking, based on number of visits, for 20 candidate attractants compared against food odor and an odorless control (deionized water) in tests with ricefield rats (three males and three females per pair compounds).

Candidate attractant	Percent response (mean ± S.E.)
Soybean oil	94.6 ± 23.0
Isovaleric aldehyde	94.3 ± 21.6
N-butyldiethanolamine	87.5 ± 38.6
N-proplyamine	80.0 ± 56.4
Peanut oil	80.0 ± 18.3
2-Furaldehyde	77.5 ± 31.3
Linseed oil	76.2 ± 24.1
Ethyldiethanolamine	75.6 ± 11.5
Sassafrass oil	74.4 ± 23.9
Dihydroxyethylaniline	72.5 ± 58.8
Wintergreen oil	72.2 ± 16.9
Corn oil	67.5 ± 16.3
Hexanoic acid	65.9 ± 25.9
N-octylamine	61.9 ± 26.6
N-amylamine	60.0 ± 42.9
Isobutylamine	52.4 ± 19.7
Cod liver oil	50.0 ± 19.3
N-(n-propyl)-benzylamine	40.0 ± 28.1
Valerone	36.4 ± 4.5
N-hexylamine	35.6 ± 3.6

The primary use of the taste tester is for a precise evaluation of such solid food materials as baits and bait carriers. It has been used to assess candidate taste enhancers and to determine the palatability of various grain-based bait carriers such as wheat, oats, rice, corn, etc., to wild Norway rats. Toxicants with acceptability problems may be examined with this device in order to determine whether the repellency is due to taste effects of physiological aversion. One limitation is that the testing of highly odorous materials may cause preference determinations to be less sensitive. However, the principle on which the tester is based has shown broad applicability to a number of species. The device as described here has been successfully used with laboratory, wild, Norway, and ricefield rats and with Peromyscus. At this conference, Campbell and Bullard will describe a preference tester for deer based on the same system; a similar device, using tubes of blood or plasma, has proved successful in tests with vampire bats (Desmodus rotundus).

The odor tester also offers improvements over previously used methods. One principal advantage is that no training or orientation period is required, since the design utilizes the typical behavior pattern of rats when exposed to an open field enclosure, that is, to explore the peripheral surfaces. In this process, the rat has the opportunity to sample each odor before preference behavior is recorded. The fact that wild rats can be used as readily as laboratory rats in such a situation means that test results should be more applicable to rodent control than results obtained with operant-conditioning techniques such as those described by Long and Tapp (1968). Since visual, auditory, and gustatory cues have been eliminated, the apparatus does not tend to promote positional bias. The use of relatively inert materials such as Teflon, glass, and stainless steel along with two water sprayers greatly facilitates cleaning odor residues after each subject is tested and thereby adds to its usefulness for screening large numbers of compounds. Through the use of a wild rodent transfer cage for entrance and exit from the odor preference tester, handling and associated stress are minimized.

The main application of the odor preference tester is to assess the reinforcing strength of odors in terms of their ability to lure rodents to baits. One of its major limitations is that precise control of the odor stimulus is not possible. Odors tend to become mixed in the open field area, and simultaneous testing of several highly odorous materials may result in poor sensitivity. Candidate attractants of both biological and nonbiological origin have been evaluated with the odor tester, but there are other possibilities for its use. With odors of biological origin but unknown chemical composition (pheromones, for example), the odor tester may be used in behavioral bioassay for isolation and identification. Concelvably, repellents as well as attractants could be tested, or the relative contribution of odor cues to sublethal aversion could be assessed for toxicant research.

In summary, both of these preference testing devices have advantages over commonly used screening methods. Both are automated, eliminating the interference and variability that would result from an operator manipulating the choice presentation. Both produce preference determinations in relatively little time (20-30 minutes per animal), and neither requires special training of test animals. Both give two simultaneous measurements of preference--number of choices and consumption in the taste tester, and number of visits and time spent near the odor in the odor tester. Finally, uniformity tests have shown that both effectively eliminate position bias; this increases both inter-subject reliability and intra-subject sensitivity, reducing the number of tests required for preference determinations.

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