A novel cognitive palatability assessment protocol for dogs

J. A. Araujo* and N. W. Milgram*†

*Department of Pharmacology and †Division of Life Science, University of Toronto, Toronto, Ontario M1C 1A4, Canada

ABSTRACT: Assessment of canine palatability is important for both the pet food and pharmaceutical industries; however, the current palatability assessment protocols are limited in their utility. The most common technique, the two-pan test, does not control for the satiating effects of food and may not be useful for long-term palatability analysis because nutritional or caloric characteristics of the diets may interfere with the results. Furthermore, the large quantities of foods consumed may be detrimental to the health of animals that do not self-limit their food intake. The purpose of this study was to determine whether a cognitive protocol could be used to determine food palatability in dogs. Five beagle dogs were trained on a three-choice object-discrimination learning task. After establishing object preferences, the preferred object was associated with no reward, a second object was associated with the dog’s normal laboratory diet (Purina Agribrands Canine Lab Chow No. 5006; Agribrands Purina Canada, Inc., Woodstock, ON, Canada), and the third object was associated with a commercial (Hill’s P/D; Hill’s Pet Nutrition Inc., Topeka, KS) diet. In the discrimination-training phase, dogs were trained until they learned to avoid the no-reward object. They were subsequently given an additional 20 test sessions, which were used to determine food preference. In the reversal phase, which involved reversal learning, the object-food associations were modified, such that the object that was previously associated with Hill’s P/D diet was now associated with the normal laboratory diet and vice versa. Once the dogs learned to avoid the no-reward object, they were tested for an additional 20 sessions. All subjects learned to avoid the no-reward object during the initial learning, and the number of choices to the object associated with the Hill’s P/D diet was greater than the number of choices to the objects associated with the dry laboratory diet (P < 0.05) and no reward (P < 0.05), indicating a strong preference for the Hill’s P/D diet. The object preferences were reversed in only three of five dogs when the food-choice associations were reversed, although the two phases did not differ significantly from one another. The protocol in the present study provides a robust measure of food palatability and circumvents many of the limitations associated with other palatability assessment techniques. The present protocol should be useful as a replacement or adjunct to other tests of palatability, but requires further validation by comparing the assessment of more similar and novel foods directly with other palatability tests.

Key Words: Animal Behavior, Canis familiaris, Cognitive, Learning, Dogs, Palatability

Introduction

Palatability is a measure of subjective food preference and depends on taste, texture, and odor. Assessment of palatability is important for the animal pharmaceutical and pet food industries: the greater the palatability, the easier and more enjoyable the administration of the substance. Because subjects are unable to declare preferences directly, palatability assessment must be based on an objective measure in which two or more foods can be ranked on the basis of preference.

The most common method of assessing palatability in dogs is with the two-pan test, which involves comparing the consumption of two different foods (Farrell, 1984a,b; Griffin et al., 1984). This procedure allows palatability to be determined rapidly, but does not control for satiety effects or food interactions in which the presence of one food can alter the palatability of the other. A second approach employs a concurrent-schedule procedure to assess the strength of an animal’s motivation to eat (Chao, 1984; Rashotte et al., 1984). A preference of one food over another is established by
pressing one lever more frequently than the other (Rashotte and Smith, 1984). This methodology circumvents many of the limitations associated with the two-pan test, but may be less robust (Rashotte et al., 1984).

We have designed a novel cognitive palatability assessment protocol (CPAP) that utilizes procedures originally developed for assessment of canine cognitive function. The purpose of the present study was to demonstrate that a CPAP would provide a robust and reliable means of assessing palatability. The protocol uses a discrimination learning procedure in which dogs are presented simultaneously with three objects, each associated either with no reward or one of two particular test foods. They are then allowed to respond to one of the objects. With sufficient experience, the animals’ response selection should become indicative of a preference for one food over the other.

**Materials and Methods**

**Subjects**

Two male and three female intact beagle dogs (*Canis familiaris*) from our colony at the University of Toronto were used. Two dogs were between 2 and 4 yr of age, and the remaining three dogs were between 9 and 10 yr of age. All subjects had been in the colony for at least 1 yr, and all had previous experience on a variety of tests of cognitive function. The subjects were housed individually in pens measuring approximately 1.07 × 1.22 m and were fed once daily, within 5 h of palatability testing. Water was available ad libitum. Dogs were maintained on a 12-h light:12-h dark cycle and were exercised daily while their pens were cleaned. All subjects underwent regular clinical examinations and had no health problems throughout the duration of the study.

**Apparatus**

A wooden chamber based on the Wisconsin General Test Apparatus (Milgram et al., 1994) was used for palatability testing (Figure 1). Vertical stainless steel bars, covering the front of the box, provided access to the objects and test foods associated with them. Objects were presented on a sliding Plexiglas tray, which contained one medial and two lateral food wells. The test foods could be accessed by displacement of the appropriate object from above the food well. The dogs and the tester were separated by a wooden screen, which had a hinged door at the bottom, to allow presentation of the sliding tray, and a one-way mirror above, which permitted the tester to view the subject. An incandescent light attached to the front of the chamber served as the only source of light during testing.

**Food Comparisons**

Two foods were compared in this study: Purina Agribrands Canine Lab Chow No. 5006 (Agribrands Purina Canada Inc., Woodstock, ON), a dry food, which also served as the regular daily diet for all the subjects; and Hill’s Prescription Diet (P/D; Hill’s Pet Nutrition Inc., Topeka, KS), a moist dog food, intended to be highly palatable.

**Test Objects**

Two sets of test objects were used. The first consisted of a yellow margarine container, a gray diskette box, and a silver can. The second set consisted of lights that differed in hue and likely intensity; one was red, one was green, and the other was yellow. The objects were selected with the intention that dogs would be able to discriminate readily between them based on work previously done in our laboratory; the results of this study also suggested that this was the case. Each subject was tested with one of the sets for all phases of the study and no differences in learning or performance were seen between the object sets, suggesting that the dogs were able to discriminate between hues and objects equally well.

**Design**

Palatability testing was divided into four phases: a preference and association phase, a discrimination-training phase, a stabilization phase, and a reversal phase.

**Phase 1. Preference and Association Testing**. The preference test was used to determine object preferences
for the test stimuli. Dogs typically respond to their preferred object; therefore, we have used the absence of responses to the preferred object as an indication that the subjects have learned a task (Milgram et al., 1994). Three different objects were presented to the subjects for 12 trials, each associated with approximately 1 g of Hill’s P/D diet, and the number of responses to each object was recorded. The use of 12 trials during this and subsequent phases was based on the simultaneous presentation of three objects and ensured the positions of the stimuli were randomized among the three possible well positions, with all possible combinations occurring equally. Although any multiple of six trials would satisfy this condition (Figure 2), 12 trials most closely approximated our previous work, which used 10 trials (Milgram et al., 1994). The object chosen most often was considered to be the subject’s preferred object. In all subsequent testing, the preferred object was associated with no reward.

Two association days followed the preference test. The purpose of the association days was to familiarize each subject with the particular objects and the test food associated with it. By providing these association days, we intended to reinforce the object-food contingencies and possibly decrease the time required to acquire the task. On the first association day, each subject received 12 trials in which one of the nonpreferred objects was presented over the middle well containing the moist test food. On the second association day, the second nonpreferred object was presented for 12 trials covering the middle well, which contained the dry test food. On both association days, only a single object was presented, and displacement of the object was always associated with a particular test food.

Phase 2. Discrimination Training. On the discrimination-training phase, the animals were given 12 trials during each daily session, with a 30-s interval separating each trial. Each trial began with the simultaneous presentation of the three objects to the animal. In a manner identical to the preference test, the location of each object was varied in a quasi-random manner to ensure that all possible combinations of stimulus placement occurred equally during a 12-trial test session (Figure 2). The preferred stimulus, determined in the preference test, was never placed over food, and the remaining objects were always placed over the test food associated with them during the association days. A trial ended after the subject displaced one of the three objects and retrieved the food (unless they responded to the object associated with no reward).

This phase of the protocol was completed when the subject passed an a priori two-stage learning criterion based on the animal’s response to the no-reward object. The first stage required the dogs to choose their preferred object fewer than two times during one test session, fewer than four times over two consecutive test sessions, or fewer than eight times over three consecutive test sessions. To pass the second stage, the subjects were required to choose their preferred object fewer than 10 times over the three consecutive sessions subsequent to passing the first criterion. The rationale for these criteria is based on our previous work, in which the first criterion stage required subjects to respond to the correct stimulus at least 80% of the time, and the second criterion stage required subjects to respond to the correct stimulus at least 70% of the time (Milgram et al., 1994). Because neither of the two nonpreferred objects was more correct than the other, we used the reciprocal criteria with choices to the preferred object.

Phase 3. Stabilization. The stabilization phase was intended to establish the strength and reliability of dietary preferences. Subjects were tested for 20 d using a procedure identical to that during the training phase. The number of choices to each object during this phase was used to establish food preference.

Phase 4. Reversal. This test phase was instituted after completing the stabilization phase. The purpose was to determine whether the preferences remained after the objects associated with the two test foods were switched. In this phase, as in the previous phase, the initially preferred object was never associated with any food. The objects associated with the test foods, however, were switched, such that the stimulus associated with the moist test food in the earlier phases was now associated with the dry test food and vice versa. This phase included association days, a training phase, and a stability phase, as described above.

Data Analysis

The number of choices of the nonpreferred objects during the stabilization phases was used to establish the food preference. A food-preference ratio, calculated by dividing the number of choices to the object associated with the moist test food by the sum of the choices of the stimuli associated with the moist and dry test foods, was used to describe the individual data. Using
Figure 3. Individual training and stabilization data over the initial learning phase. This shows the responses to each of the three objects on each test session over the initial training and stabilization sessions for one subject. The arrow indicates the point at which the subject reaches criterion on the training phase. This dog quickly developed a preference for responding to the object associated with the moist test food (PD), and this preference was maintained over the stabilization sessions. This subject made few responses to the objects associated with no reward and the dry food (chow) during stabilization.

Table 1. Results of all subjects on initial learning

<table>
<thead>
<tr>
<th>Item</th>
<th>Subject No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sessions to criterion</td>
<td>14</td>
</tr>
<tr>
<td>Responses during stabilization trials</td>
<td>Moist food</td>
</tr>
<tr>
<td></td>
<td>No reward</td>
</tr>
<tr>
<td></td>
<td>Dry food</td>
</tr>
<tr>
<td>Preference ratio</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*aNumber of daily sessions to reach criterion.

*bPreference ratios were calculated using the formula: choices of Hill’s Prescription Diet (P/D; Hill’s Pet Nutrition Inc., Topeka, KS)/ (choices of PD + choices of chow).

Results

All subjects learned to avoid the object associated with no reward within 14 test sessions (mean = 10.6; SD = 2.5; Figure 3). During the subsequent stabilization sessions, every subject showed a very strong and reliable preference for the object associated with the moist (Hill’s P/D) diet (Table 1). Only four of the five subjects, however, reached criterion after the reversal of objects associated with the two food types. Of these four, three demonstrated an increase in choices of the object associated with the P/D diet (Figure 4). The fourth subject continued to choose the object that was associated previously with the moist test food, despite the changed reward. The overall choices of the moist test food greatly outnumbered the choices of either the dry test food or the preferred stimulus over both the initial and reversal phases. A difference between object choices was noted...
(F_{2,8} = 12.88; P < 0.01; observed power = 0.94), but not between the initial and reversal test phases. A post-hoc Fisher’s test indicated that the difference was due to a greater number of choices of the object associated with the moist test food compared with the object associated with the dry test food (P < 0.05) and the no-reward object (P < 0.05). The choices to the object associated with the dry test food and the no-reward object did not differ.

To determine whether the overall results were due to choices in the first phase, we examined the contribution of the individual phases to overall food choice. For the original learning phase, an effect of food choice was found (F_{2,8} = 21.70, P < 0.001; observed power = 0.99), but no effect was found on the reversal phase (Figure 5). A post-hoc Fisher’s test indicated that the difference was due to a greater number of choices of the object associated with the moist test food compared with the objects associated with the dry test food (P < 0.05) and the no-reward object (P < 0.05). The choices to the objects associated with the dry test food and the no-reward object did not differ.

To assess the stability of food choice over the original learning phase, the mean preference ratio scores for the first five and last five stability sessions of the original learning phase were calculated for each subject and compared using a dependent t-test. No differences were found.

**Discussion**

As hypothesized, the CPAP provided a successful method for establishing food preferences in beagle dogs. During the initial training phase, all subjects learned to avoid responding to the object associated with no reward. Over the subsequent stabilization phase, the subjects developed a very strong and stable choice preference for the object associated with the moist test food, which suggests the subjects preferred the moist test food to the dry test food. This interpretation is supported further by the response pattern on the reversal phase, where three of the five subjects reversed their object response pattern, consequently choosing the object associated with moist test food. The primary purpose of the present experiment was to demonstrate that dogs could learn to select one object over another based on taste preferences. Because the procedure might have required extensive training, we initially used only five subjects. Nevertheless, the results proved to be sufficiently robust that a statistically significant effect was obtained. The present results, therefore, indicate that a cognitive test procedure can be used to assess palatability, and also suggests that the CPAP possesses a higher level of power to detect differences in palatability than other palatability-assessment procedures.

We have used reversal learning in the past to examine an animal’s ability to modify its response pattern to two stimuli. In the present study, we used the reversal-learning paradigm to confirm that animals were responding based on food and not to a particular object. The initial palatability measure was established by demonstrating a learned preference toward approaching one object over two others, and every animal showed the same preference. However, the results were more variable when the foods associated with the two objects were reversed. Three of the five dogs changed their preference to the new object associated with the P/D diet, a fourth dog maintained its previous object preference, and the fifth animal was unable to pass the learning criteria. This increase in variability is consistent with our previous studies of discrimination reversal learning, in which it typically takes longer to learn the reversal than it does the initial discrimination (Milgram et al., 1994; Tapp et al., 2003). The new association must not only be learned, but the old association must be broken. Another factor that probably affected the variability in the reversal task was age. We have shown previously that discrimination reversal learning is impaired in aged vs. young dogs (Milgram et al., 1994; Tapp et al., 2003), but most individuals in both groups are capable of succeeding in the task. In the present study, the two dogs that did not demonstrate a change in object choices were both greater than 9 yr of age, which we have previously classified as old (Milgram et al., 1994; Tapp et al., 2003). We have also demonstrated that dogs in this age range are cognitively impaired relative to younger dogs (Milgram et al., 1994; Adams et al., 2000; Chan et al., 2002; Tapp et al., 2003). Three of the subjects, however, switched object preference in accordance with the preferred diet. Considering that responses to the object initially associated with the moist food in the initial learning phase were rewarded with the dry food in the reversal phase, the finding that some subjects altered their response further supports...
the hypothesis that animals were responding based on food type and not to a particular object.

Given the limited number of subjects used in the present study, several factors may have influenced the results. As we mentioned above, age likely contributed to the findings in the reversal phase. Although further studies are required to examine possible age effects on food preference using the CPAP, the original learning sufficiently indicated the preferred food and suggests that the reversal phase is not necessary to establish food preferences. This was supported by the highly significant and powerful findings on the initial stabilization phase of the study, but not on the reversal. In fact, the addition of the reversal phase increased the length of the study and the variability of the results. Therefore, the use of the reversal phase in practical applications of the CPAP is questionable and likely not required. Gender also may have influenced the findings in the present study; however, we did not find gender differences in discrimination learning and reversal in a previous study (Tapp et al., 2003). Lastly, the objects used may have influenced the results because we previously found differences in learning using objects of various complexities (Tapp et al., 2003). Because the rate of learning the tasks was not used in the assessment, it is unclear to what extent these factors may have influenced performance in the present study. Further, the limited subject number did not permit us to readily determine the influence of these factors in the present study. The overall observed power was high, however, which suggests that these factors and the limited number of subjects did not impact the palatability results substantially. Further studies examining these factors are warranted, but likely will require a larger number of subjects to discern any differences if the food preference differences are as large as in the present study. It also is unclear, based on the literature, how age and gender affect the assessment of palatability using other techniques.

Performance on the two-pan test can be influenced by a variety of noncognitive factors, including satiety and the possibility that the palatability of one food affects the preference for the other. The cognitive protocol controls for these factors by using a forced-choice paradigm and by limiting the amount of food consumed. Furthermore, this novel approach to palatability testing can be varied to examine short- or long-term preferences without confounding nutritional or caloric effects; the large amount of food consumed using the two-pan test may decrease its utility in long-term testing (Sunday et al., 1983) and may be unhealthy for animals that do not self-limit their food intake. Other factors that also can be studied with a CPAP include pre- and post-feeding differences, temporal differences, and changes induced by hormonal or pharmacological treatment.

The main advantage of the two-pan test over the CPAP is the speed of testing. As we mentioned above, the use of the reversal phase is not necessary to establish food preference, which will decrease the time required for palatability assessment with the CPAP. Furthermore, acquisition of the task could be facilitated with massed training, in which additional trials are given daily. Although we used 20 stabilization days to assess food preference, preferences established over the first five sessions did not differ from the last five sessions; thus, the number of stabilization days could also be reduced. These additional modifications should decrease the amount of time necessary to adequately assess food preference using the CPAP and make the procedure more applicable to the pet food industry. Additional experiments, however, are required to validate these modifications and compare the utility of the CPAP with that of the two-pan test.

Rashotte and Smith (1984) outline the advantages of using a concurrent-schedule procedure to test palatability over the two-pan test. These include providing good measures of hedonic values that can be used to form a hedonic scale, the use of small quantities of food that minimize postingestional influences, and using the number of lever presses as a scale to compare foods that differ in ways not easily quantifiable. Compared with the CPAP described here, none of these is a characteristic unique to the concurrent-schedule protocol. Furthermore, the CPAP is more readily learned, which may be due to the proximity of the object-food associations as the proximity of associations affects learning rate (D’Mello and Steckler, 1996).

The present paradigm involved a single forced choice, which differs from both the concurrent-schedule paradigm and the two-pan test, in which both foods can be obtained concurrently. In fact, it is the development of a side bias during a test session that indicates food preference in the concurrent-schedule paradigm and the two-pan test. Typically, dogs will attempt to obtain as much food as possible, which may confound the preference data obtained. This is particularly important when the foods do not differ greatly in palatability and may explain why testing foods with various fat contents or testing moist and dry foods does not provide either graded or strong preference scores (Rashotte et al., 1984). Another notable feature of the CPAP used here is the use of a no-reward object. The absence of responses to the no-reward object indicates learning and provides an essential control for the possibility of an animal having no preference between the two foods.

A remaining issue in evaluating different procedures for assessing palatability is that of test sensitivity; to what degree must two foods differ to obtain reliable results? Rashotte and Smith (1984) argue that the concurrent-schedule paradigm permits the study of preferences in foods that are similar in composition. The procedure, however, was insensitive to differences in fat content. In addition, preference ratios were typically small. For the best responder, the preference ratio was approximately 80%, even when the foods were substantially different, such as dry vs. semimoist foods. By contrast, three of the five animals tested using the CPAP had preference scores above 80% in the initial
stabilization phase, suggesting greater test sensitivity. This is further supported by the presence of significant results with the use of only five subjects.

One factor that may have contributed to the very high preference scores obtained in the present study was the use of the standard laboratory chow as one of the foods. We attempted to control this by separating the test and feeding times by at least 18 h. Another possibility is that the difference in texture, which is also an important feature of palatability, between the test foods was responsible for the large preference differences in the present study. Subsequent investigations comparing the CPAP with the other palatability tests should compare foods that are more similar in texture, appearance, and odor, and that are novel to the subjects to control for any effects of previous food experience. Findings from such studies, demonstrating greater power to detect differences with the CPAP compared with other palatability-assessment techniques, would support the replacement of other palatability-assessment techniques with the CPAP.

The purpose of this study was to determine whether a CPAP could be used to test the palatability of two test foods in dogs. The results clearly demonstrate that dogs are able to associate a particular object with a preferred food. These results also demonstrate that a CPAP can be used to test palatability, and therefore, to establish food preferences in dogs. This approach provides an objective measure of food preference using a limited number of animals, while controlling for other factors influencing feeding, such as satiety. Not only does this task offer advantages over the traditional two-pan task, its ease and speed make it more appealing than a concurrent-schedule test. Furthermore, the cognitive test protocol can easily be modified to examine the contribution of other factors, such as age, hormonal state, and dietary experience, to canine food preference. Future studies to validate the CPAP should examine the effects of age, gender, and food experience and should compare the sensitivity of the CPAP with other palatability assessment techniques. Although further studies are required to determine the utility and practicality of this procedure for the pet food industry, the high level of significance and power achieved in the present study suggest the CPAP may be useful as either a replacement or as an adjunct to other currently used palatability test protocols.

**Literature Cited**


