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Systematic Assessment of Food Item Preference and Reinforcer Effectiveness: Enhancements in Training Laboratory-Housed Rhesus Macaques

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Abstract

The use of systematic preference assessments can enhance positive reinforcement training with captive animals. We found that the multiple stimulus without replacement (MSWO) technique identified food preferences in laboratory housed rhesus macaques, with raisins and grapes being ranked higher on average than dried apricot, pasta, and green beans (Friedman Test, $\chi^2(4) = 35.52, p < .001$). Agreement between individuals ($N = 21$) was moderate (Kendall's $W = .42$), and consistency across time varied among individuals ($W = .03$ to $.90$). Highly preferred items identified by the MSWO assessment were subsequently found to increase subjects' engagement in a husbandry task on which they were being trained (Mann-Whitney $U = 6.00, p = .002$) and to improve performance on a progressive ratio schedule (Wilcoxon signed-rank test, $Z = -2.17, p = .03$) when compared with low preference items. The progressive ratio technique supplements other preference assessment techniques by measuring the amount of work a subject will do to gain access to an item. The use of more effective reinforcers identified through systematic assessment has the potential to increase animal performance on husbandry and research tasks and to improve animal welfare in the laboratory setting.

Keywords

animal training; animal welfare; positive reinforcement training; preference testing; progressive ratio; reinforcer effectiveness

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1. Introduction

Food rewards are widely used as part of positive reinforcement training procedures with nonhuman primates in the laboratory setting, both for experimental testing procedures such as cognitive touch screen tasks (e.g., Crofts et al., 1999; Scott et al., 2003; Tu & Hampton, 2014) and for voluntary cooperation with research or husbandry tasks (e.g., Coleman et al., 2008; McMillan et al., 2014; Veeder et al., 2009). The use of positive reinforcement animal training within behavioral management programs has grown tremendously in the last two decades (Laule et al., 2003; Perlman et al., 2012) and is now included in standards of care set forth by the *Guide for the Care and Use of Laboratory Animals* (National Research Council of the National Academies, 2011). However, this type of training involves a considerable time investment and relies on the voluntary participation of animals, which can be challenging to achieve for unpleasant tasks such as those that involve discomfort (e.g., presenting for an injection). The use of highly preferred food rewards that serve as effective reinforcers may increase the efficiency and effectiveness of these training procedures.

In addition, research tasks such as those used in some behavioral neuroscience experiments often require reliable and stable performances over a large number of trials or on complex tasks. In these cases, food or fluid restriction is sometimes used to improve performance, and this restriction can have a negative impact on animal wellbeing (see Prescott et al., 2010). Prescott et al. (2011) and Westlund (2012) suggest that taking individual animals' food preferences into account may allow a reduction in the use of fluid and food restriction in laboratory-housed rhesus macaques, resulting in improved welfare for these animals.

1.1 Identifying Preferred Stimuli

Commonly, food rewards or other stimuli are chosen as potential reinforcers based on convenience or on the perceptions of trainers as to which foods may be preferred by the animal. However, these choices have been found to have low correlation with empirically-determined preferences in both animals (Gaalema et al., 2011; Mehrkam & Dorey, 2015) and humans (Cote et al., 2007; Green et al., 1988). For humans, empirically-determined preferred items were found to function as reinforcers in applied tasks whereas those identified by caregivers were not (Green et al., 1988), indicating the importance of empirically-determined preferences. In addition, just because a food (or other stimulus) is preferred does not necessarily mean that it will serve as an effective reinforcer for a particular behavior. Thus, one must distinguish between food preference assessments that determine which foods an animal might consume first or in higher quantities and reinforcer assessments that determine whether the delivery of a specific food will effectively increase a target behavior.

Researchers have used a variety of methods to identify preferred stimuli in both humans and animals. In humans, the majority of preference research has been conducted to determine effective reinforcers in clinical settings for children with developmental disabilities (e.g., Carr et al., 2000; Pace et al., 1985) or in educational settings (e.g., Mintz et al., 2007). In animals, food preference assessments have been conducted for a variety of reasons, including nutritional/palatability assessments (e.g., Tobie et al., 2015), comparative studies (e.g., Remis, 2002), measurements of cognitive capacity (e.g., Benz et al., 1992), pest

control (e.g., Morgan, 1990), and a few assessments aimed at determining possible training reinforcers in applied animal settings (e.g., Clay et al., 2009; Fernandez et al., 2004; Gaalema et al., 2011). These preference assessments can be broadly categorized based on how the items are presented to the subject; whether they are presented one at a time, in pairs, or in groups.

1.2 Types of Preference Assessments

Single-item assessments involve presenting stimuli one at a time, and the dependent measure is some gauge of subject interaction with the stimulus, such as whether a child approaches a stimulus (Pace et al., 1985) or whether an animal consumes a food item (Cameron et al., 2013). These single-item assessments are relatively easy to conduct, but they can be time consuming and are sensitive to both ceiling and satiation effects (Fisher et al., 1992; Polidora & Schneider, 1964). For example, a child might approach each toy that is presented, resulting in no differentiation (Fisher et al., 1992).

For these reasons, preference assessments involving choice between multiple items have become more popular. Based on concurrent operant paradigms (Catania, 1963, 1966; Herrnstein, 1970), subjects exposed to the paired-choice paradigm must choose one of two simultaneously presented items. This procedure is repeated until all stimuli have been paired with all other items in the array, and items that are chosen a high percentage of the trials in which they are available are considered highly preferred (Fisher et al., 1992). Fisher et al. (1992) found that this forced-choice procedure resulted in greater differentiation among stimuli and also was a better predictor of which stimuli would subsequently serve as effective reinforcers. Paired-choice procedures are widely used to determine food, toy, or activity preferences in humans with developmental disorders (e.g., DeLeon et al., 2001; Fisher et al., 1992; Piazza et al., 1996) and have also been used to determine food preferences for a variety of animal species, ranging from great apes to possums (e.g., Benz et al., 1992; Cameron et al., 2013; Clay et al., 2009; Fernandez et al., 2004; Gaalema et al., 2011; Harlow & Myer, 1952; Mehrkam & Dorey, 2014, 2015; Polidora & Schneider, 1964; Remis, 2000; Vicars et al., 2014).

Finally, in the multiple-stimulus without replacement (MSWO) procedure (DeLeon & Iwata, 1996), several items are presented concurrently. When a subject chooses an item from the array, the array is taken away and the subject gets access to the chosen item for a short period of time. The remaining items (minus the previously chosen item) are then re-presented, and the subject makes another choice. This is repeated until all items have been chosen or until the subject stops responding. For this assessment, preference is determined by the order in which items are selected. Developed for use with developmentally disabled humans, MSWO procedures were found to be more time-efficient than paired-choice procedures, and the results were found to be similar to the paired-choice technique in rank and consistency (DeLeon & Iwata, 1996).

An additional advantage of the MSWO procedure is that its brevity (generally only taking 1 – 2 minutes to conduct) allows clinicians to conduct frequent preference assessments. Preferences have been found to vary across time and context in both humans (DeLeon et al., 2001; Kennedy & Haring, 1993; Mason et al., 1989) and animals (Clay et al., 2009).

DeLeon et al. (2001) used a pre-treatment, paired-choice procedure to identify initial preferences in developmentally disabled individuals. Then, the researchers used daily, brief, MSWO procedures to identify preferences for those same items just prior to each work session. When daily preferences differed from initial preferences, the preferred items identified by the daily sessions were found to serve as more effective reinforcers than the initially preferred item, indicating a benefit in conducting frequent preference assessments just prior to work sessions.

Both paired-choice and MSWO techniques rely on choice behavior (i.e. pointing to or approaching an item), which requires minimal effort with little response cost, and thus may not be an accurate predictor of whether the items identified will be effective as reinforcers for more demanding or high-cost behaviors (DeLeon et al., 1997; Tustin, 1994). Thus, techniques that measure the amount of work a subject will do to gain access to an item may provide additional information about the effectiveness of that item as a reinforcer for more complex tasks such as those used in applied training settings (Roane et al., 2001). For example, some animal studies identified preferred foods by comparing the rates at which animals performed some basic task (e.g., pressing levers, pecking keys) to gain access to a variety of food items (e.g., Boyer et al., 1974; Bruce et al., 2003; Hovland et al., 2006; Polidora & Schneider, 1964; Treichler et al., 1966).

An extension of this type of operant paradigm is the progressive ratio technique. The progressive ratio technique was originally developed by Hodos (1961) to find stimuli that served as suitable reinforcers for animals. In a progressive ratio schedule, the response requirement to earn access to a reinforcer increases by some increment on a trial by trial basis. That is, in order to gain access to a stimulus, a subject may have to emit one response, but in the next trial the subject may have to emit that same response two times to gain access to the same stimulus, and so on. The session is terminated after a response is not given during a predetermined amount of time. Progressive ratio analyses can provide some insight into the potency of a stimulus; that is, how long a reinforcer can maintain a response that requires a high amount of effort (Roane, 2008). This potency can be assessed by looking at the break point or the last schedule completed in a progressive ratio assessment (Roane, 2008). Results of the progressive ratio schedules generally support findings of other types of stimulus assessments. Stimuli identified as highly preferred through preference assessments (e.g., paired-choice procedures) have subsequently been shown to maintain higher rates of responding under increasing progressive ratio schedule requirements than items that were considered less preferred in both human and non-human populations (Cameron et al., 2015; Cameron et al., 2016; DeLeon et al., 2009; Hutson & van Mourik, 1981; Kennedy & Baldwin, 1972; Penrod et al., 2008; Vicars et al., 2014). Furthermore, progressive ratio assessments have been beneficial in showing differential responding with stimuli that may appear equally preferred in a preference assessment (Roane et al., 2001).

1.3. Assessing Reinforcer Efficacy

Once a method is identified for successfully identifying preferences among foods or other stimuli, the next step is to determine whether the use of high preference items results in a meaningful increase in task performance in applied settings. Applied behavior analysts have

investigated the effects of these preferences on task motivation and performance in developmentally disabled humans. Items identified as highly preferred using the various preference assessments were found to improve performance on a variety of adaptive tasks including increasing compliance with verbal requests to complete basic tasks such as raising a hand or repeating certain words, increasing in-seat behavior, and improving puzzle completion (Carr et al., 2000; DeLeon & Iwata, 1996; Fisher et al., 1992; Pace et al., 1985; Piazza et al., 1996). This demonstrated the usefulness of these procedures to not only identify preferences, but also to identify stimuli that can function as effective reinforcers in applied settings.

However, there has been limited animal research on how preferences translate into reinforcer effectiveness. In the few studies that have investigated this, the tasks involved have been relatively basic. For example, Gaalema et al. (2011) show that items identified as highly preferred during a paired-choice procedure were also differentially selected during a subsequent concurrent choice task (i.e. touching or pushing various stimuli) in giant pandas (*Ailuropoda melanoleuca*) and African elephants (*Loxodonta Africana*). Similarly, Vicars et al. (2014) found that items identified as highly preferred by a paired-choice task in domestic dogs (*Canis lupus familiaris*) subsequently served as effective reinforcers in a progressive ratio procedure in which the task was touching the experimenter's fist with the dog's nose. However, it is unknown whether items identified by preference techniques will translate to faster learning or more efficient training sessions when training animals for more complex tasks.

1.4. Study Aims

The current investigation consisted of three experiments. In the first experiment, we investigated whether MSWO procedures would successfully identify food preferences in laboratory housed rhesus macaques (*Macaca mulatta*) and whether those preferences would differ between subjects and across time. We then evaluated the ability of preferred food items identified in the MSWO assessment to subsequently serve as effective reinforcers in both an applied husbandry task (Experiment 2) and a progressive ratio task (Experiment 3).

2. General Materials and Methods

2.1. Subjects

A total of 24 female rhesus macaques (*Macaca mulatta*) were used in the three studies. All subjects were housed at the Yerkes National Primate Research Center (YNPRC, Atlanta, GA). Subjects ranged in age from 5.3 – 22.6 years ($M = 13.8$, $SD = 7.5$) at the start of the study. The subjects were assigned to a project assessing aging in primates. Because of this assignment, animals fell into two age groups: young (5–10 years old) and old (older than 19 years old). Thus, we also analyzed data for the effect of age on our training tasks.

2.2. Housing

The animals were housed in adjoining 4.3 to 6 cubic foot cages. The majority of animals ($n = 22$) were pair-housed throughout the study period. Two subjects were singly housed during portions of the study due to social incompatibility. All subjects received water ad libitum and

were given food and environmental enrichment in accordance with federal regulations and YNPRC standard operating procedures. This included twice-daily primate chow, regular distribution of fresh produce, access to a perch and toy at all times, and intermittent access to many other types of enrichment. Training and testing for this project was performed in the animals' home cages. The YNPRC is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All work was approved by the Emory University IACUC and followed the guidelines outlined in *The Guide for the Care and Use of Laboratory Animals* (National Research Council of the National Academies, 2011)

2.3. Materials

Foods used during training included grapes, raisins, dry elbow macaroni, green beans, and dried apricots. All foods were prepared so that they were approximately the size of a raisin. For the MSWO assessment, the foods were placed on an 8.5cm x 18.2cm plastic tray with shallow (.5cm) divided wells to hold the food items. Other materials included a training clicker, appropriate personal protective equipment, a clock/timer, and two training targets. The hanging target consisted of a polyvinyl chloride (PVC) plastic elbow joint connected to a chain that was hung on the cage front using a snap hook. The hand target consisted of a golf ball attached to the end of a plastic dowel.

2.4. Data Collection and Analysis

Data were collected on paper data sheets and analyzed using IBM SPSS[®] version 24.

3. Experiment 1: MSWO Preference Assessment

The purpose of this study was to evaluate the MSWO protocol to assess food preferences in rhesus macaques. We hypothesized that this technique would show preference differentiation among the tested food items. Preference assessments were conducted across a one-year time period to determine the stability of food preferences across time.

3.1 Method

3.1.1 Subjects—Of the original 24 subjects, sufficient MSWO data for analysis were available on 21 animals (10 in the old age category, and 11 in the young age category). MSWO preference assessments were conducted prior to each training session in which the animal was in a high or low preference condition, but not for sessions in which an animal was in a random food condition (see Experiment 2). Due to the random assignment of conditions, we had two subjects who were always in the random condition and thus did not participate in any MSWO assessments. A third animal's data were excluded because she only received four MSWO preference assessments over three days. All other animals had MSWO assessments conducted over a period of at least six months. We considered her data insufficient to examine her preference stability across time.

3.1.2 Procedures.—Brief MSWO preference assessments were conducted prior to training sessions over an average of 12.6 months ($SD = 2.8$, range = 6.7 – 18.1). Training sessions focused on a variety of tasks required for the aging study, including voluntary social

separation (training data presented in Experiment 2) and subsequent research tasks including vaginal swabbing and blood collection (training data not presented).

For the MSWO procedure, the five food items were arranged quasi-randomly on the plastic tray and placed at the front of the monkey's cage. A choice was recorded any time the monkey made physical contact with a food item. The monkey was then given a few seconds to consume the chosen food item, and the remaining items were rearranged and represented. This procedure was repeated until all food items were chosen. If the monkey did not make a choice within 30 seconds of the tray presentation, the tray was removed and presented again. If after 3 presentations no choice was made, the trial was scored as "no choice."

3.1.2. Inter-observer reliability.—Data were collected on the order of items chosen. For inter-observer reliability purposes, a second researcher observed and independently scored 12.9% of all MSWO assessments. An agreement was scored if both observers recorded selection of the same stimulus during the trial. A disagreement was scored if observers recorded different stimuli. Inter-observer agreement was calculated by dividing the number of agreements by the number of agreements plus disagreements and converting this ratio to a percentage. Mean percent agreement was 95.9%.

3.1.3. Data Analysis.—A nonparametric Friedman test was used to determine whether the median rankings of the five food items differed significantly. Kendall's W tests (also known as Kendall's coefficient of concordance) were calculated to determine both the consistency of preferences across individuals and the consistency of preferences across time within each individual monkey. In addition, a Mann-Whitney Test was used to examine the relationship between age and food preference stability. For all analyses, the alpha level was set at .05. For the purpose of data analysis, if two or more items were not chosen (i.e. scored "no choice" in the MSWO), those data were entered as ties. For example, if a monkey only made three choices, the remaining two items were both given a score of 4.5.

3.2. Results

A mean of 66.9 MSWO sessions per subject were conducted ($SD = 32.0$, range = 15 – 124). Median rankings of the five food items differed significantly (Friedman Test, $\chi^2(4) = 35.52$, $p < .001$), with raisin as the highest ranked food item and green bean as the lowest (Average median rankings: raisin = 2.00, grape = 2.60, apricot = 2.90, pasta = 3.33, green bean = 4.17). Agreement between animals was calculated using the Kendall's W statistic, which ranges from 0 (no agreement) to 1 (perfect agreement). Our subjects showed moderate agreement on the five food items (Kendall's $W = .42$).

Kendall's W statistics were also used to quantify the consistency of each monkey's food rankings across time. Consistency across time ranged from very low (Kendall's $W = .03$) to very high ($W = .90$, see Figure 1). The Kendall's W statistics across all individuals were approximately normally distributed (Shapiro-Wilk = .948, $p = .308$) and averaged .36 ($SD = .24$). There was no statistically significant difference in consistency across time between young ($n = 11$, mean Kendall's $W = .27$, $SD = .22$) and old ($n = 10$, mean Kendall's $W = .46$, $SD = .07$) subjects, Mann-Whitney $U = 30$, $p = .078$.

3.3. Discussion

The MSWO procedure identified clear food preferences in our 21 rhesus macaque subjects, with raisins and grapes being ranked higher on average than dried apricot, pasta, and green bean. Agreement between individuals was moderate (Kendall's $W = .42$), indicating that there was variation in rankings among individuals. This underlines the merit of conducting individual preference assessments rather than relying on species-level generalizations about which foods an animal might prefer.

For each subject, we conducted numerous MSWO assessments across a period of approximately one year, allowing us to examine the stability of each subject's choices across time. Out of our 21 subjects, eight showed low consistency (Kendall's $W < .3$) across time, nine showed moderate consistency ($.3 < W < .6$), and only four showed high consistency (Kendall's $W > .6$). This finding is consistent with previous studies showing changes in preferences across time (Clay et al., 2009; DeLeon et al., 2001; Kennedy & Haring, 1993; Mason et al., 1989) and illustrates the need for frequent retesting of preferences for maximum accuracy. Unlike the paired-choice tests used in previous animal studies, the ease and brevity of the MSWO procedure allows for frequent reassessments, ideally conducting a preference assessment just prior to a training session, increasing the effectiveness of this technique in identifying highly preferred items that may also serve as effective reinforcers.

4. Experiment 2: Social Separation

The purpose of this study was to determine whether the use of highly preferred food items (as identified by the MSWO procedure) would result in more efficient training of a training task. The training task we studied was social separation. Each of the social pairs shared two adjacent cages. However, the aging study included a touch screen task that required the animals to be singly housed during the brief testing sessions. Thus, training was initiated to teach the animals to voluntarily move to separate cages and allow the insertion of a temporary, solid panel between the cages prior to the touch screen activity. We hypothesized that highly preferred foods would increase subjects' engagement with the training task and decrease the amount of time required to train social separation.

4.1. Method

4.1.1. Subjects.—Data from two of the original 24 subjects were excluded from this analysis. One subject was not trained on social separation due to social incompatibility. An additional subject was trained with a subject who had received previous separation training. Having an experienced partner may have aided her acquisition of the task, so her social separation training data were excluded. However, her engagement scores during the task were recorded and used in that analysis.

4.1.2. Procedures.—Prior to the study, all animals were acclimated to the clicker by repeated pairings of the click with delivery of a food item. In addition, prior to social separation training, all animals were trained using positive reinforcement training (i.e. a click/treat combination delivered contingent on successive approximations to the final behavior) to touch both the hanging target and the hand target on cue.

Animals were assigned using random block (by age) assignment to one of three groups: high preference ($n = 4$ pairs), low preference ($n = 4$ pairs), and random ($n = 3$ pairs). In the random condition, the trainer used a random number generator to select one of the five stimuli to be used during the sessions. An MSWO preference assessment was conducted prior to each social separation session for monkeys in the high or low preference condition. Monkeys in the high preference condition were given their first consumed choice from the MSWO assessment. Monkeys in the low preference condition were given their last consumed choice from the MSWO assessment.

During social separation training, pairs were trained using positive reinforcement training to move to the appropriate cage, hold the target hung on the front of their cages, and remain in position while a panel was inserted between the two cages. Animals were considered trained on this behavior after three consecutive sessions where the monkeys remained in position and allowed the panel to fully close. Data were collected on the reinforcer used during the session, the level of training achieved for each training session (i.e. separated, separated with panel), the duration of the training session, and engagement score. The engagement score was based on a scale of 1–4 with 1 reflecting no engagement and 4 reflecting maximum engagement (see Table 1 for engagement score definitions).

4.1.3. Interobserver reliability.—A second experimenter observed and independently scored 20.3% of all social separation training sessions. Inter-observer reliability averaged 100% for reinforcer used, 96.61% for session duration, 94.9% for training criteria reached, and 94.8% for engagement score.

4.1.4. Data analysis.—Due to the cooperative nature of the social separation task, measures related to training success were analyzed at the pair level ($N = 11$). However, training engagement was analyzed at the individual level ($N = 23$). One pair in the low preference condition still had not met training criteria after 32 training sessions (158 minutes of total training time). To expedite training, that pair was then switched to the high preference condition. For the purpose of this analysis, only data from their first 32 sessions were used, and their training time was recorded as 158 minutes.

We used Kruskal-Wallis tests (the nonparametric equivalent of the one-way analysis of variance) to determine whether preference condition (high, random, or low) had a significant effect on training time and engagement score. We also examined the effect of individual age (old/young) on engagement score using a Mann-Whitney U test (the nonparametric equivalent of a t-test), and we examined the effect of pair age (young, mixed, or old) on training time using a Kruskal-Wallis test. The relationship between engagement score and training time was analyzed using a nonparametric Spearman's Rho correlation. Alpha levels were set at .05.

4.2. Results

Overall, it took an average of 52.8 minutes to train the social separation task ($SD = 54.5$, range = 4 – 158 min). There was no significant effect of preference condition (high $M = 40.25$, $SD = 61.30$; random $M = 43.67$, $SD = 26.31$; or low $M = 72.23$, $SD = 69.85$) on training time (Kruskal-Wallis test, $\chi^2(2) = 1.30$, $p = .52$). The age of the pair also did not

significantly affect training time (young $M = 69.20$, $SD = 50.01$; mixed $M = 85.00$, $SD = 103.24$; old $M = 16.25$, $SD = 9.43$) Kruskal-Wallis test, $\chi^2(2) = 1.75$, $p = .42$).

Preference condition (high, random, or low) did have a significant effect on engagement scores (Kruskal-Wallis test, $\chi^2(2) = 10.00$, $p = .007$). Bonferroni-corrected, post-hoc, pairwise comparisons using Mann-Whitney U tests showed that animals in the high preference condition ($n = 9$) had significantly higher engagement scores ($M = 3.73$, $SD = .40$) than animals in the low preference condition ($n = 8$, $M = 3.07$, $SD = .28$, Mann-Whitney U = 6.00, $p = .002$). The engagement score for animals in the random condition fell between these two values ($n = 6$, $M = 3.36$, $SD = .38$) but did not differ significantly from either the high or low preference animals. There was no significant effect of age on training engagement, with young animals ($n = 12$) having similar engagement scores ($M = 3.23$, $SD = .37$) as old animals ($n = 11$, $M = 3.56$, $SD = .49$, Mann-Whitney U = 40.00, $p = .118$). There was also a significant, negative correlation between an individual animal's engagement score and their training time, indicating that those with higher engagement scores learned the training task faster than those with lower engagement scores (Spearman's rho = $-.638$, $p = .001$).

4.3. Discussion

Our goal in conducting this experiment was to test whether the use of food items identified as highly preferred by an MSWO would reduce training times and increase cooperation with a complex training task. In hindsight, the social separation task was perhaps not the best task to use for this demonstration. The willingness of animals to separate from one another likely has many influences, including the nature of the relationship between the two animals in each pair. In addition, because of the cooperative nature of the task, our dependent measures had to be analyzed at the pair level, limiting our statistical power. Thus, perhaps it is not a surprise that we did not find a significant effect of food preference condition on training times. We recommend further studies on how the use of highly preferred food items will affect performance on other, applied, complex training tasks.

More encouragingly, we did find that those animals who were given their preferred food item as reinforcers during training sessions were more engaged in their training sessions than were animals given their low preference food item. Valuable training time is lost when subjects are not engaged in the training task (e.g., focusing their attention on other tasks, leaving the training area) which can lead researchers to consider fluid or food restriction (Prescott et al., 2010). We found a negative correlation between our subjects' engagement score and the time it took for them to learn the training task. The use of highly preferred food items has promise in making training sessions more efficient and perhaps in reducing the use of fluid or food restriction.

5. Experiment 3: Progressive Ratio

Given our mixed results in the social separation task, we wanted to further explore whether food items identified as highly preferred by the MSWO protocol would subsequently serve as effective reinforcers in a more controlled study. We hypothesized that animals would work harder for highly preferred items than they would for less preferred food items. We

also examined the relationship between choice consistency and task performance, hypothesizing that individuals showing more consistent choices across time (i.e., having stable, highly preferred items as opposed to variable preferences) would also show a bigger difference in performance when compared between high and low preference conditions. For this experiment, we chose a more basic, individual task of touching a target as a measure of the animal's willingness to work for various food items and measured this using a progressive ratio task.

5.1. Method

5.1.1. Subjects.—Of the original 24 subjects, 3 subjects died prior to the start of progressive ratio testing, leaving 21 subjects (12 young and 9 old) for this assessment.

5.1.2. Procedures.—Monkeys were given a MSWO preference assessment immediately prior to the beginning of each progressive ratio (PR) session. All monkeys completed four PR sessions, two in the high preference condition and two in the low preference conditions. In the high preference condition, monkeys were given their first choice from the MSWO assessment, and in the low preference condition, monkeys were given their last choice from the MSWO assessment. The order of these conditions was randomized, and a maximum of one session was conducted per day.

Because the targeting task used in the PR assessment was one of many trained behaviors for these subjects, a warm-up, task familiarization exercise was conducted just prior to each PR session to orient the monkey to the targeting task. During this familiarization task, each monkey was prompted to touch the hand target five times, with reinforcement (i.e. click/treat combination) provided on a fixed ratio (FR) 1 schedule. A touch was defined as any part of the hand coming into contact with the ball at the end of the dowel. Once this warm-up exercise was complete, the PR assessment began.

The goal of the PR assessment was to determine how many responses, or how much work, the monkeys would perform for their high and low preference food items. On the first trial of the PR assessment (PR=1), the target was presented at the front of the monkey's cage with the verbal cue "target." After one touch of the target, the trainer clicked and provided a food item. During each subsequent trial, the number of times the monkey had to touch the target to receive access to the food stimulus increased by one. For example, on the second trial, the criteria for reinforcement was two target touches (PR=2). After each trial was completed, the target was removed and re-presented with the cue "target." Monkeys were given time to consume the item before the next trial began. The response requirement was increased until the animal reached its limit of responding for the item being tested. This is called a "break point" in a progressive ratio task, and we defined this as the animal exhibiting no target touches for two minutes. At that point, the session was terminated. Data were collected on session duration, the number of times the subject touched the target during a given ratio schedule requirement, and the last progressive ratio schedule completed during each session.

5.1.3. Interrater reliability.—A second, independent observer collected data during 11.9% of all progressive ratio sessions. Inter-observer agreement was 99.9% for total

number of responses per trial, 100% for last progressive ratio requirement completed, and 100% for session duration.

5.1.4. Data Analysis.—To assess the influence of high versus low preference items on the progressive ratio task, each animal's performance on her two high preference sessions was compared with her performance on her two low preference sessions. Wilcoxon signed-rank tests (a nonparametric alternative to the paired t-test) were used to compare the mean last progressive ratio schedule completed and mean response rates (responses per minute) between the high and low preference conditions. The relationship between age and progressive ratio performance was analyzed using Mann-Whitney U tests. To investigate the influence of preference consistency on performance on the PR task, we first found the difference between the mean last progressive ratio schedule completed in the high and low preference conditions. Then, for the 19 subjects who had data from both experiments, we correlated that difference score with each individual's Kendall's W score from Experiment 1 using a Pearson product-moment correlation coefficient. Alpha was set at .05 for all tests.

5.2. Results

Animals worked more (as measured by mean last progressive ratio schedule completed) for high preference food items ($M = 18.14$, $SD = 7.49$) than for low preference food items ($M = 15.45$, $SD = 5.47$, Wilcoxon signed-rank test, $Z = -2.17$, $p = .03$). Animals also had higher response rates under the high preference condition ($M = 6.76$, $SD = 2.17$) as compared with the low preference condition ($M = 5.88$, $SD = 1.79$, Wilcoxon signed-rank test, $Z = -2.55$, $p = .01$).

When all sessions were combined, younger animals ($n = 12$) completed more progressive ratio schedules ($M = 19.71$, $SD = 5.95$) than older animals ($n = 9$, $M = 12.92$, $SD = 3.77$, Mann-Whitney U = 18.50, $p = .009$). Response rates were similar between young ($M = 6.51$, $SD = 1.60$) and old ($M = 5.81$, $SD = 1.84$) monkeys (Mann-Whitney U = 41.00, $p = .356$).

There was no correlation between an individual's choice consistency (Kendall's W from Experiment 1) and difference score between the mean last progressive ratio schedule completed in the high and low preference conditions, $r(17) = -.26$, $p = .27$.

5.3. Discussion

Our rhesus macaque subjects conducted more work and worked more quickly when provided with a highly preferred food item as compared with a less preferred food item. Our study comparing MSWO results with progressive ratio performance extends research conducted in both the human and non-human literature where stimuli that were more preferred in a paired choice preference assessment served as better reinforcers under increasing response requirements (Cameron et al., 2015; Cameron et al., 2016; DeLeon et al., 2009; Penrod et al., 2008; Hutson & van Mourik, 1981; Vicars et al., 2014). Progressive ratio assessments provide an efficient means of extending the findings of preference assessments by quantifying how well a reinforcer will continue to maintain a response when more effort is needed. This is important as some behaviors that may be trained could require

a great deal of effort on the part of the subject or may have some aversive qualities (e.g., injection training).

No relationship was found between an individual monkey's consistency of choice and their difference in performance between the high and low preference conditions of the PR task. If a difference had been found, it could have indicated that those animals showing low choice consistency were simply indifferent to the choices (and thus had similar performances in both PR conditions) whereas those who made consistent choices had stronger preferences (resulting in a bigger difference in performance). The lack of a relationship between these variables may suggest that the preferences in those animals with low choice consistency may be just as strong as those with high choice consistency but that their preference simply varies more frequently.

6. General Discussion

Taken together, our results show that the MSWO procedure was effective in identifying food preferences in rhesus macaques, and that those items identified as highly preferred subsequently served as effective reinforcers in an applied task. Animals given highly preferred food rewards were more engaged in the training sessions for social separation than animals given lower preference food items. In addition, animals receiving food items contingent on touching a hand target performed better on a progressive ratio schedule when they received highly preferred foods compared with when they received lower preferred food items.

Our subjects showed variation in preferences between individuals. This accentuates the importance of preference testing at the individual (rather than just species) level to ensure that effective reinforcers are being used in positive reinforcement training sessions. In addition, most of our subjects showed a great deal of variation in their preferences across time which suggests the need for frequent reassessment of preferences to maximize the benefit to positive reinforcement training programs. The MSWO procedure is easy and quick to perform, allowing preferences to be assessed just prior to each training session, and we suggest primate trainers can incorporate this step into their training routines.

The incorporation of preference testing into positive reinforcement training sessions has the potential to do more than simply increase training efficiency. It may also convey welfare benefits. For example, in children with developmental disabilities, the use of preferred reinforcers as determined by systematic assessment not only increased accuracy in tasks but also reduced the children's maladaptive behaviors during the teaching sessions (Mason et al., 1989). In animal studies, the use of highly preferred items as reinforcers may also decrease the need for food restriction. Bruce et al. (2003) found that using highly preferred maggots as food reinforcers for an operant task in laying hens resulted in similar motivation as approximately 9 hours of food deprivation. Westlund (2012) proposed that the use of preference tests may also result in a reduction in the need for fluid and food restriction in laboratory-housed primates. Finally, given the fact that nonhuman primates have been shown to prefer choice conditions (Perdue et al., 2014), the simple act of choosing a reinforcer may, in itself, provide a welfare benefit to animals.

Our work demonstrates that the MSWO procedure is an efficient method for determining preferences in rhesus macaques and that items identified by this method serve as effective reinforcers for training tasks. Positive reinforcement training has already improved animal husbandry practices and animal welfare in captive environments, and the incorporation of systematic preference assessments into this training represents a next step in the use of applied behavior analytic principles in this setting (Maple & Segura, 2015). Future work could build on our findings by examining how other behavior analytic principles could be used to increase reinforcer effectiveness. For example, in her commentary, Westlund (2012) encourages the examination of the effect of conditioned reinforcement and variable-ratio schedules on the reinforcer effectiveness. In addition, human clinical work has shown that individuals show a preference for varied over constant reinforcers (Bowman et al., 1997), and using varied reinforcers increased correct responding and on-task behavior in children with developmental disorders (Egel, 1981). Wilson et al. (2005) have developed an automated food delivery system that can dispense a variety of foods to monkeys, allowing an opportunity to study this effect in laboratory primates.

6.1 Conclusions

The application of a MSWO assessment technique identified preferences for food items among our rhesus macaque subjects. Agreement between individuals was only moderate, which underscores the value of conducting the individual preference assessments rather than relying on species-level generalizations about which foods an animal might prefer. Rhesus macaques were more engaged in training sessions when their more preferred reinforcers were used as compared to when their low preference reinforcers were used. Subjects worked more and worked more quickly when provided with a highly preferred food item as compared to a less preferred food item, as measured by a progressive ratio assessment. Conducting systematic preference assessments has the potential to enhance positive reinforcement training with captive animals.

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Highlights

- A multiple stimulus assessment identified food preferences in rhesus macaques.
- The consistency of food preferences across subjects and time was variable.
- Using preferred foods increased engagement during husbandry training sessions.
- Using preferred foods increased performance in a progressive ratio task.

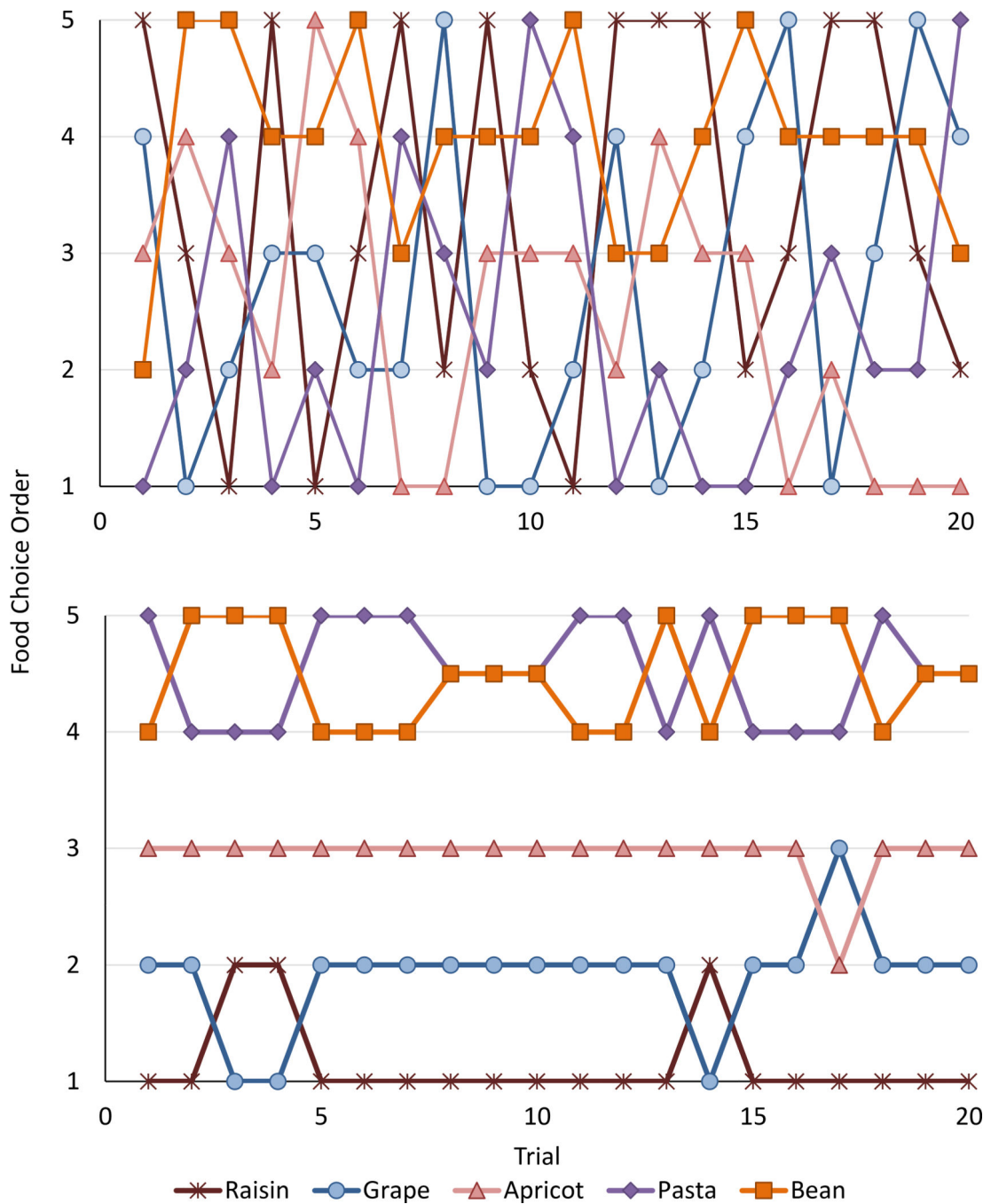


Figure 1. A comparison of choice consistency in the multiple stimulus without replacement (MSWO) task for a subset of trials ($n = 20$) in two subjects. The subject illustrated in the top panel had low choice consistency across time (overall ($N = 37$) Kendall's $W = .03$), and the subject illustrated in the bottom panel had high choice consistency across time (overall ($N = 42$) Kendall's $W = .90$).

Table 1:

Engagement Score. An engagement score of 1–4 was given to each subject for each training session based on the following criteria:

1: No Engagement	Animal does not approach the cage front, does not accept food rewards, and does not engage in social interaction with trainer
2: Minimal Engagement	Animal approaches cage front at least briefly and accepts food rewards or engages in social interaction with trainer but does not comply with any training command or offer any behaviors.
3: Moderate Engagement	Animal approaches and stays at cage front for some duration, complies with some known training commands or offers approximations toward behaviors in progress. However, the training session also includes periods of disengagement where animal leaves the cage front or causes interruptions in training due to aggression, etc.
4: Maximum Engagement	Animal approaches cage front, complies with most training tasks, and remains engaged for the duration of the training session.