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Effects of inter-food interval on the variety effect in an instrumental food-seeking task. Clarifying the role of habituation[☆]

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Abstract

Food variety increases consumption and the rate of instrumental behavior that is reinforced by food in humans and animals. The present experiment investigated the relationship between the variety effect and habituation to food by testing the role of the interval between successive food presentations on responding in an operant food-seeking task. Habituation to food was expected at short, but not long, interfood intervals. The effects of variety on food's long-term reinforcing value were also tested. Four groups of rats were trained to lever-press on different random-interval (RI) schedules of reinforcement to earn 45-mg food pellets. Half the rats in each group received an unpredictable mix of grain and sucrose pellets, while the other half consistently received sucrose pellets. Response rate began at a high rate and then decreased within each 30-min session for groups that received short inter-pellet intervals (i.e., RI-3 s and RI-6 s reinforcement schedules) but not in groups that received longer inter-pellet intervals (i.e., RI-12 s and RI-24 s). A variety effect in the form of higher responding in the mix group than the sucrose-only group was also only evident at the shorter intervals. Habituation and variety effects were also most evident with the short intervals when we controlled for the number of reinforcers earned, suggesting that they were not merely due to rapid satiation. The variety effect also appeared quickly when groups trained with longer inter-pellet intervals (RI-12 s and RI-24 s) were transitioned to shorter intervals (RI-3 s and RI-6 s). There was no effect of variety on resistance to extinction or on resistance to the response-suppressing effects of pre-session feeding. The results more clearly link this version of the variety effect to the short-term effect of variety on food habituation.

Keywords

Food habituation; Variety effect; Reinforcement rate; Behavioral persistence

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Introduction

Appetitive behavior is stronger when organisms receive a variety of foods than when they receive the same food repeatedly (e.g., Raynor & Epstein, 2001; Remick, Polivy, & Pliner, 2009). This *variety effect* has been demonstrated in humans and animals when investigators have measured either food consumption or the rate of operant food-seeking behavior that is reinforced by food. One explanation of the variety effect is that variety slows habituation that can otherwise develop as a consequence of repeated presentations of the same food. *Food habituation* describes the reduction in eating that occurs as an eating episode progresses. When organisms are given the same food repeatedly, responding for it decreases (e.g., Epstein, Temple, Roemmich, & Bouton, 2009). Habituation may contribute to the cessation of eating within a meal. Variety may slow this process for several reasons (Bouton, Todd, Miles, León, & Epstein, 2013). For example, because habituated responding can “dishabituate” after a new stimulus (e.g., a new food type) is presented, providing one type of food might dishabituate suppressed responding to another food. Alternatively, because habituation is stimulus-specific, responding might recover whenever the food is changed. In addition, because habituation is slower when the habituating stimulus is distributed more widely in time, variety might slow habituation by increasing the interval between successive presentations of the same food. Any or all of these facts could contribute to a variety effect. However, we are not aware of any evidence to confirm that the variety effect occurs because of variety’s specific effect on habituation. The purpose of the present research was thus to further examine the relationship between the variety effect and habituation to food.

The variety effect has been primarily studied in humans (Ernst & Epstein, 2002; see Epstein et al., 2009, for review). However, Bouton et al. (2013) recently studied the effect in an operant food-seeking task in rats. Using a method introduced by Aoyama and McSweeney (2001), they gave rats the opportunity to earn a 45-mg food pellet for every 4th lever press they performed in each of a series of daily 30-min sessions. After several sessions of training, responding began at a high rate at the start of each session and then declined through the remainder of the session, a result that was consistent with the possibility that the effects of the food pellet habituated within each session. Consistent with a variety effect, Bouton et al. found that presenting an unpredictable sequence of sucrose- and grain-based pellets slowed the decline in response rate that was otherwise observed when either pellet was presented exclusively. The effect became stronger over repeated sessions, as the within-session decline in responding deepened. That result is consistent with the possibility that the effect of variety on instrumental responding was due to its effect on habituation. However, enhanced responding for the mixture was also evident early in training, before the within-session decline became substantial. The fact that the phenomenon was evident so early leaves the possibility open that variety might also increase the reinforcing value of food in some manner that is separate from its effects on habituation. Although the results of at least one other study with animal subjects also suggests that variety can enhance responding in an operant task involving within-session decreases in responding (Lupfer-Johnson, Murphy, Blackwell, LaCasse, & Drummond, 2010), we are not aware of any evidence to suggest that food habituation is *necessary* to observe the variety effect.

Interestingly, Bouton et al. (2013, Experiment 2) also isolated a second “variety effect” that was not linked to within-session habituation. When rats were given alternating sessions that contained grain or sucrose pellet reinforcers consistently, the amount of responding for sucrose during sucrose sessions exceeded that observed in a control group that only earned sucrose pellets in every session. That effect was attributed to incentive contrast (e.g., Flaherty, 1996); exposure to a highly palatable food after exposure to a less palatable food can increase its positive effects. In this sense, variety can enhance responding between-sessions by virtue of a mechanism that is different from habituation. It is worth noting, however, that Bouton et al. (2013) also observed less responding to the grain pellet in the alternating group than in a group that received grain pellets consistently. Thus, the two pellets produced both positive and negative incentive contrast, and the average level of responding across sucrose and grain sessions was not different from that of the control animals.

The present experiment was designed to explore the role of habituation in producing the effect of variety on within-session responding in more detail. Most importantly, it was designed to separate variety’s immediate effects on habituation from the possibility that it also has an impact on food’s longer-term reinforcing value. As in the experiments of Bouton et al. (2013), rats lever-pressed to earn food pellets over a series of 30-min sessions. Different groups received either consistent sucrose pellets or an unpredictable mixture of sucrose and grain pellets. If anything, the present sucrose pellets are weakly preferred to the present grain pellets (Bouton et al., 2013; Winterbauer, Lucke, & Bouton, 2013). As a result, any demonstration of increased responding for the mixture of grain and sucrose pellets over sucrose pellets alone would provide a compelling demonstration of the variety effect.

To explore the role of habituation, different groups also earned the pellets at different rates: Pellets could be earned on Random Interval (RI) schedules of reinforcement that delivered pellets for the first response emitted after intervals averaging either 3, 6, 12, or 24 s since the last food pellet. The use of RI schedules, as opposed to the fixed ratio schedule used before (Bouton et al., 2013), provided better experimental control over the rate at which pellets were encountered. Because habituation generally occurs more quickly when the habituating stimulus is presented at high rates (e.g., Rankin et al., 2009), we expected that within-session habituation would be most pronounced with the higher rates of reinforcement. The question was whether the variety effect would also be most evident at those rates. If variety affects response rate by influencing the habituation process, a variety effect should be observed primarily in groups that otherwise demonstrate within-session habituation.

The experiment also included tests designed to assess whether variety affected behavioral persistence over and above its effect on within-session responding. After training with sucrose only or the sucrose/grain mixture, responding was tested (1.) during extinction, when pellet delivery was discontinued, and (2.) after satiation produced by free access to food immediately before a test session. If variety makes foods more reinforcing, it might increase behavior’s resistance to extinction and/or resistance to satiation. Resistance to extinction and the effects of satiation have been considered indices of the reinforcer-produced “momentum” of operant behavior (Nevin & Grace, 2000).

Method

Subjects

Forty-eight naïve female Wistar rats purchased from Charles River Laboratories (St. Constance, Quebec) participated in the study. They were between 75 and 90 days old at the start of the experiment and were individually housed in suspended wire-mesh cages in a room maintained on a 16:8-hr light:dark cycle. Rats were maintained at 80% of their free-feeding body weights via small daily feeding of the maintenance chow, P500 Prolab RMH 3000 (PMI Nutrition International, Brentwood, MO).

Apparatus

The apparatus consisted of two unique sets of four operant conditioning chambers (Med Associates, St Albans, VT, model ENV-008-VP). All boxes measured 30.5 × 24.1 × 23.5 cm (length × width × height). The floor was made of stainless steel grids (0.48-cm diameter) and the ceiling and sidewalls were made of clear acrylic plastic. The front and rear walls were made of brushed aluminum. A recessed 5.1 cm × 5.1 cm food cup was centered in the front wall 2.5 cm above the floor. In both sets of boxes, a retractable lever (4.8 cm long and positioned 6.2 cm above the floor grid) was positioned 7.8 cm (center to center) to the right of the food cup. When extended, the lever protruded 1.9 cm from the front wall. Two 28-V panel lights (2.5 cm in diameter) were attached to the wall 10.8 cm above the floor and 6.4 cm to the left and right of the food cup. Ventilation fan provided background noise of 65 db.

The two sets of conditioning chambers had unique features that allowed them to be used as different contexts (Bouton et al., 2013; counterbalanced), although they were not used in that capacity here. In one set of four chambers, one acrylic plastic sidewall had black diagonal stripes, 3.8 cm wide and 3.8 cm apart. The ceiling had similarly spaced stripes oriented in the same direction. The floor grids were spaced 1.6 cm apart (center to center) on the same plane. The other set of boxes had no distinct visual cues, the floor grids were spaced 1.6 cm apart (center to center) and staggered such that odd-and even-numbered grids were mounted in two separate places, one 0.5 cm above the other. Each chamber in both sets of boxes was illuminated by one 7.5-W incandescent bulb mounted to the ceiling of the conditioning chamber, 34.9 cm from the grid floor, near the back wall.

There were two pellet reinforcers, both obtained from Test Diet, Richmond, IN, USA. One was a 45-mg grain-based food pellet (MLab Rodent Tablet [5TUM]), and the other was a 45-mg sucrose pellet (Sucrose Tablet [5TUT]). The different pellets were delivered by separate feeders that delivered the pellets to the same food cup. Previous research in this laboratory indicates little generalization between the two types of pellets or between the pellets and homecage chow (e.g., Bouton et al., 2013). According to manufacturer specifications, each of these food types was identical in caloric density (3.4 kcal/g) but differed in macronutrient composition. Specifically, the percentages of calories from protein, fat, and carbohydrates were (respectively) 69.8%, 30.2%, and 0% for the grain pellets; 0%, 0%, and 100% for the sucrose pellets; and 36%, 14%, and 60% for the chow.

The apparatus was controlled by a computer located in an adjacent room. Sessions were conducted 7 days per week at approximately the same time each day.

Procedure

The experiment was conducted over a series of daily sessions that were always 30 min in duration unless noted otherwise.

Magazine training

On Day 0, all rats received magazine training during a single 30-min session. Approximately 60 pellets were delivered to the rat according to a random-time 30-s schedule. The type of pellets delivered in magazine training was determined by group membership; rats that would receive a mixture of grain and sucrose pellets received a random mixture at this time, while rats that would only receive sucrose pellets received only sucrose pellets. Levers were retracted (not available) during the magazine training session.

Response training and habituation

Lever press training began on Day 1. The subjects were randomly assigned to eight groups. Each group received pellets for lever pressing according to a random-interval (RI) schedule. Groups were assigned according to a 2 (reinforcer type) by 4 (reinforcement schedule) factorial design. Two groups each experienced one RI schedule, arranging the availability of a pellet for a response in a given second with a 1 in 3, 1 in 6, 1 in 12, or 1 in 24 probability. Groups therefore experienced either RI 3-s, 6-s, 12-s, or 24-s, respectively. Each RI schedule group was assigned to receive either sucrose only or a mixture of grain and sucrose. Thus, Group RI-3 s Suc ($n = 6$) received a sucrose pellet for the first lever press that occurred an average of 3 s since the last pellet was earned. Group RI-3 s Mix ($n = 6$) received either a sucrose or a grain pellet ($p = .5$) for lever pressing (with the restriction that no more than four pellets of the same type could be earned consecutively) according to the same RI-3 s schedule. Group RI-6 s Suc ($n = 6$) received a sucrose pellet according to a RI-6 s schedule, in which the next pellet could be earned (on average) when 6 s had elapsed since the last pellet, and Group RI-6 s Mix ($n = 6$) received a mixture of grain and sucrose according to the same RI-6 s schedule. In a similar way, Groups RI-12 s Suc and RI-12 s Mix ($n = 6$) received either sucrose pellets or a mixture of grain and sucrose pellets (respectively) according to a RI-12 s schedule, and Groups RI-24 s Suc and RI-24 s Mix ($n = 6$) received either sucrose pellets or a mixture of grain and sucrose pellets according to a RI-24 s schedule. In each session, 2 min after the rat was placed in the chamber, the lever was inserted and the reinforcement schedule was in effect until the lever was retracted 30 min later (Bouton et al., 2013). Hand shaping was not necessary. Response training continued for 12 consecutive days.

Extinction and extended sessions

Because of the pattern of data that developed during response training and habituation, groups in the RI-3 and RI-6 s conditions and the RI-12 s and RI-24 s conditions were given different tests on Day 13. Groups RI-3 s and RI-6 s, which both demonstrated a variety effect, were given an extinction test to see whether the stronger responding evident in the Mix groups would be sustained when food pellets were discontinued. Each of these groups received a 30-min session in which responses were recorded but had no scheduled consequences. Because Groups RI-12 s and RI-24 s had shown little evidence of either

habituation or a variety effect, they were given an extended session in which they could earn pellets according to their usual schedules for a total of 120 min. The idea was to give them enough accumulated exposure to the pellets to allow responding to decline and reveal a possible variety effect.

Retraining

On Days 14–17, Groups RI-3 s and RI-6 s were returned to their original reinforcement schedules. Half of the animals in each of the RI-12 s and RI-24 s groups now received RI-3 s or RI-6 s, counterbalanced; individual rats continued to receive either sucrose only or the sucrose/grain mix contingency that they had received before.

Satiation test

On Day 18, half of the rats in each group, counterbalanced for schedule history, were given ad lib access to their maintenance chow for 30 min prior to a test session. The other rats received no food, but were handled equivalently. Training contingencies that were in effect on Days 14–17 remained operative during the 30 min session for all groups.

Data analysis

All results were evaluated with analysis of variance (ANOVAs) using $p < 0.05$ as the rejection criterion.

Results

Within-session patterns of food-seeking during training

Responding over time within the sessions—The results of the response training phase are summarized in Fig. 1, which shows responses over the 30 1-min intervals of the session averaged over the final six sessions of training. Each panel presents responding for the Mix and Sucrose-only groups for a given RI schedule. The figure suggests that within-session decreases in responding were most evident in the groups given the shorter inter-pellet intervals (RI-3 s and RI-6 s). There was relatively little evidence of a decrease with the longer intervals (RI-12 s and RI-24 s). In addition, the Mix produced more responding than sucrose-only at the shorter intervals. Somewhat surprisingly, whereas the increased responding for the mixture was evident early in the session in the RI 3-s groups, it tended to develop later in the session for the RI 6-s groups.

These observations were examined with a Reinforcement Schedule (4) by Reinforcer Type (2) by Minute (30) repeated-measures ANOVA. The analysis confirmed a significant main effect of schedule, $F(3, 40) = 10.43$, $MSE = 2035.86$, $p < .001$, but no main effect of reinforcer type, $F(1, 40) = 1.06$, or a reinforcer type by Schedule interaction, $F(3, 40) = 2.03$. There was a significant effect of Minute, $F(29, 1160) = 31.14$, $MSE = 16.73$, $p < .001$, and a significant Schedule $\times$ Minute interaction, $F(87, 1160) = 9.08$, $p < .001$, but no Reinforcer Type $\times$ Minute interaction, $F(29, 1160) < 1$. However, there was a significant three-way interaction, $F(87, 1160) = 2.31$, $p < .001$.

To investigate the three-way interaction, we performed ANOVAs that compared mixture and sucrose-only responding over minutes in each interval grouping. For RI-3 s, there was a significant effect of reinforcer type (mix or sucrose), $F(1, 10) = 8.51$, $MSE = 219.78$, $p = .015$, and Minute, $F(29, 290) = 37.75$, $MSE = 16.27$, $p < .001$, and a Reinforcer Type by Minute interaction, $F(29, 290) = 1.89$, $p = .005$. For RI-6 s, there was also a significant effect of reinforcer type, $F(1, 10) = 5.29$, $MSE = 1994.50$, $p = .04$, and Minute, $F(29, 290) = 14.27$, $MSE = 22.29$, $p < .001$, as well as a Reinforcer Type $\times$ Minute interaction, $F(29, 290) = 2.90$, $p < .001$. For RI-12 s, there was no effect of reinforcer type, $F(1, 10) < 1$, $MSE = 1878.22$, and although responding decreased over Minutes, $F(29, 290) = 2.36$, $MSE = 14.96$, $p < .001$, minutes did not interact with reinforcer type, $F(29, 290) = 1.29$, $p = .15$. Finally, for RI-24 s, there was no effect of reinforcer type, $F(1, 10) < 1$, $MSE = 4050.95$, Minute, $F(29, 290) < 1$, $MSE = 13.41$, or a reinforcer by minute interaction, $F(29, 290) < 1$. Thus, with the possible exception of the RI 12-s groups, where there was a significant but barely-visible habituation effect, the variety effect was mostly represented in groups that also demonstrated habituation.

Further analysis also compared the RI-3 s and RI-6 s conditions. A Reinforcement Schedule by Reinforcer Type by Minute ANOVA confirmed an overall main effect of reinforcer type (variety), $F(1, 20) = 9.62$, $MSE = 1107.14$, $p = .006$. In addition, a Schedule main effect, $F(1, 20) = 27.75$, $p < .001$, indicated that the rats responded faster in the RI 6-s condition. However, there was no schedule $\times$ reinforcer interaction, $F(1, 20) = 1.59$, $p < .22$. There was a significant main effect of Minute, $F(29, 580) = 45.79$, $p < .001$, that did not interact with reinforcer type, $F(29, 580) < 1$. However, responding over minutes interacted with reinforcement schedule, $F(29, 580) = 2.57$, $p < .001$, and the three-way interaction was also significant, $F(29, 580) = 4.42$, $p < .001$. The three-way interaction is consistent with the impression given by Fig. 1 that although habituation- and variety-effects were observed with both the RI-3 s and RI-6 s schedules, the variety effect was greater at the beginning of the session with RI-3 s, but developed over the course of the session with RI-6 s.

Responding as a function of reinforcers earned instead of time—One

complication in looking at responding as a function of time is that rats given the different reinforcement schedules were accumulating food pellets at vastly different rates. Therefore, the decline of responding over minutes in the shorter-RI conditions could have been due to factors besides habituation that resulted from the rapid accumulation of reinforcers (e.g., rapid filling of the gut or rapid drying of the mouth). To better isolate habituation, Fig. 2 plots the groups' average response rates over blocks of ten reinforcers, calculated as the number of lever presses emitted within each block of ten pellets divided by the time required to earn each block of ten pellets. Like Fig. 1, the data were averaged over the final six sessions of training. The figure truncates after 6 blocks (60 earned reinforcers), because animals in the RI-24 s groups did not always earn more than this (the maximum they could earn in a session was approximately 75 reinforcers).

Despite the different way of analyzing the data, the pattern is similar to that in Fig. 1. Once again, the decline in responding mostly occurred in rats given the shorter RI intervals, where the variety effect was also the most apparent. A Reinforcement Schedule (4) by Reinforcer Type (2) by 10-Reinforcer Block (6) ANOVA revealed a significant effect of Reinforcement

Schedule, $F(3, 40) = 2.83$, $MSE = 525.71$, $p = .05$, but no main effect of reinforcer type, $F(1, 40) = 1.38$, or a reinforcer type $\times$ schedule interaction, $F(3, 40) = 1.94$. The block effect was highly significant, $F(5, 200) = 12.02$, $MSE = 11.23$, $p < .001$, as was the block $\times$ schedule interaction, $F(15, 200) = 4.77$, $p < .001$. There was no block by reinforcer type interaction, $F(5, 200) < 1$, although there was once again a three-way interaction, $F(15, 200) = 2.70$, $p = .001$.

To explore the three-way interaction, separate ANOVAs were again conducted for each reinforcement schedule. For RI-3 s, there was a main effect of reinforcer type, $F(1, 10) = 7.88$, $MSE = 348.98$, $p = .019$, and block, $F(5, 50) = 5.09$, $MSE = 12.60$, $p < .001$. The interaction was not significant, $F(5, 50) = 1.70$, $p = .29$. For RI-6 s, there was no effect of reinforcer type, $F(1, 10) < 1$, $MSE = 594.09$, but there was a significant block effect, $F(5, 50) = 6.62$, $MSE = 13.13$, $p < .001$, as well as a reinforcer type $\times$ block interaction, $F(5, 50) = 4.54$, $p = .002$. For RI-12 s, there was an effect of block, $F(5, 50) = 6.15$, $MSE = 12.22$, $p < .001$, but no effect of reinforcer type, $F(1, 10) < 1$, $MSE = 370.80$, or an interaction, $F(5, 50) = 1.34$, $p = .27$. Finally, for RI-24 s, there was also an effect of block, $F(5, 50) = 2.71$, $MSE = 6.96$, $p = 0.03$, but no effect of reinforcer type, $F(1, 10) < 1$, $MSE = 788.98$, or interaction, $F(5, 50) = 1.12$, $p = .37$. As before, the variety effect was mostly evident in animals given the 3-s and 6-s inter-pellet intervals. The lack of a reinforcer type $\times$ block interaction in the RI 3-s group was not due to the truncated range of data analyzed (six blocks of 10 reinforcers). Even when response rates were analyzed over 20 blocks of 10 reinforcers, significant effects of reinforcer type, $F(1, 10) = 8.08$, $MSE = 910.87$, $p = .017$, and block, $F(19, 190) = 23.69$, $MSE = 14.38$, $p < .001$, were observed, and the interaction remained non-significant, $F(19, 190) = 1.08$.

A subsequent analysis that isolated the RI-3 s and RI-6 s conditions for comparison found a main effect of reinforcer type, $F(1, 20) = 5.92$, $MSE = 471.53$, $p = .02$, but no effect of schedule or a reinforcer type by schedule interaction, $F_s(1, 20) < 1$. There was a main effect of block, $F(5, 100) = 12.73$, $MSE = 12.87$, $p < .001$, which interacted with reinforcement type, $F(5, 100) = 5.03$, $p < .001$. Block also interacted with reinforcement schedule, $F(5, 100) = 2.94$, $p = .02$, but there was no three-way interaction, $F(5, 100) < 1$. The lack of three-way interaction here, and the presence of one when we analyzed responding over time, suggests that the previous three-way interaction was due to the very rapid rate at which the RI-3 s animals received their pellets (which was corrected here).

Search for sensitization effects

Sensitization is defined as an increase in responding over time that is sometimes observed during early exposures to the habituating stimulus (e.g., Epstein et al., 2003; McSweeney, Hinson, & Cannon, 1996). Figures 1 and 2 suggest that there was little evidence of sensitization in the behavior of any of the groups. This state of affairs was also confirmed when we looked for evidence of sensitization within individual subjects. To identify sensitization, we modified a classification system utilized in previous work on sensitization and habituation by Epstein, Rodefer, Wisniewski, and Caggiula (1992). Rats in each group were categorized as “sensitizers” or “non-sensitizers” based on their responding in the early portions of the final acquisition session. According to one criterion, we isolated animals that

responded more in minute 2 than in minute 1. This identified 1–2 rats per group as sensitizers, regardless of schedule or reinforcer type. Rats that met this criterion consistently began the session with fewer responses in the first minute than rats whose response rates contrastingly decreased. And after an initial increase in response rate, they performed identically to the others in the group and did not show further increases. In a second criterion, we classified animals as sensitizers based on whether or not response rate increased from the first to the second reinforcer presentation. This criterion identified 2–3 animals in each group as potential “sensitizers;” once again, there was no variation according to schedule or reinforcer type. The same was true when we identified rats whose rate increased over 1st, 2nd, and 3rd reinforcers in the session. Overall, the data do not encourage the view that sensitization plays a role in the current method, or that different inter-pellet intervals or reinforcer types support different levels of sensitization. We should note, however, that the *ns* in this experiment ($n=6$) might have not provided sufficient power to detect a small sensitization effect.

Extinction in the RI-3 s and RI-6 s groups

Did the variety effect evident in the RI-3 s and RI-6 s groups produce a long-term strengthening of lever pressing? To assess the possibility, these groups underwent extinction on Day 13. The left panels of Fig. 3 present their responding over 1-min bins throughout the extinction session. There was no evidence of different rates of extinction. A RI Schedule (2) by reinforcer type (2) by bin (30) repeated-measures ANOVA found a significant effect of bin, $F(29, 580) = 4.68$, $MSE = 48.97$, $p < 0.001$, but no effects of reinforcer type or schedule (largest $F = 2.02$, $MSE = 851.09$), and no other effects or interactions (largest $F = 1.22$). An ANOVA comparing Mix and Sucrose in RI-3 s found that responding in both groups decreased over minutes of the session, $F(29, 290) = 5.01$, $MSE = 35.91$, $p < 0.001$, but there were no effects of reinforcer type or a reinforcer by minute interaction $F_s < 1$. A similar ANOVA comparing the RI-6 s groups also found that responding decreased during the session, $F(29, 290) = 1.76$, $MSE = 62.03$, $p = .01$, but no effect of reinforcer type or a reinforcer type $\times$ minute interaction, $F_s < 1$.

To further compare extinction performance in these groups, the right panels of Fig. 3 show average response rates from the extinction session and the last preceding session for groups RI-3 s and RI-6 s. A repeated-measures ANOVA comparing overall response rates in each schedule and reinforcer type across the two sessions found greater response rates in RI 6-s, $F(1, 20) = 12.68$, $MSE = 67.04$, $p = 0.002$, but no main effect of reinforcer type, $F(1, 20) = 3.63$, $p = .07$, and no schedule by reinforcer type interaction, $F < 1$. Not surprisingly, there was overall less responding in extinction than the last baseline session, $F(1, 20) = 57.18$, $MSE = 48.44$, $p < .001$. This effect interacted with prior reinforcement schedule, $F(1, 20) = 7.03$, $p = .015$, but not with reinforcer type, $F(1, 20) = 3.63$, $p = .07$. The three-way interaction was also not significant, $F(1, 20) = 1.17$, $p = .29$. We also expressed each animal's response rate in extinction as a proportion of its rate in the last baseline session. Here there were no significant effects of schedule, reinforcer type, or a schedule by reinforcer type interaction, all $F_s < 1$. Thus, although the grain/sucrose mixture produced more responding than sucrose alone during training, there was no evidence that it created more behavioral persistence in extinction.

Extended test in the RI-12 s and RI-24 s groups

Figure 4 presents average responses over four minute bins in the two-hour testing session for groups responding on RI-12 s and RI-24 s that occurred on Day 13. This test addressed the question of whether the sucrose/grain mixture would begin to produce a variety effect in these groups if given an opportunity to earn more pellets than they earned in the usual 30-min sessions. A Schedule (2) by reinforcer type (2) by 4-min bin (30) repeated-measures ANOVA found no effects of schedule, reinforcer type, or an interaction, $F_s < 1$. Responding did decrease in rate over the session, as indicated by an effect of bin, $F(29, 580) = 29.73$, $MSE = 111.54$, $p < .001$. There was also a schedule by bin interaction, $F(29, 580) = 4.12$, $p < .001$, which took the form of a slower decline in responding in the RI-24 s rats. However, no other two-or three-way interactions approached significance, $F_s < 1$. While extending the session thus led to faster habituation and/or satiation in the RI-12 s groups, there was no reliable evidence of a variety effect in either group.

Figure 5 shows response rates in this test plotted as a function of 10-reinforcer blocks. Response rates for the first 200 reinforcers are presented (rats in RI-24 s condition began dropping out after that point). The figure suggests little change in response rate as a function of accumulating reinforcers, and again no evidence of a variety effect. A schedule (2) by reinforcer type (2) by block (20) repeated-measures ANOVA found no effects of schedule, $F(1, 20) = 3.34$, $MSE = 5467.07$, $p = .08$, reinforcer type, $F < 1$, or schedule by reinforcer type interaction, $F < 1$. There was a significant effect of block, $F(19, 380) = 2.78$, $MSE = 69.09$, $p < .001$, but here there were no interactions with block, largest $F = 1.34$, $p_s > .1$. There was thus no evidence of a variety effect when the RI-12 s and RI-24 s groups were given an extended session. The variety effect may thus be linked rather exclusively to higher reinforcement rates (RI-3 s and RI-6 s).

RI-12 S and RI-24 s groups reassigned to RI-3 s and RI-6 s

Following the two-hour testing session, half of the rats in the RI-12 s and RI-24 s groups were each allowed to respond on either RI-3 s or RI-6 s for an additional four 30-min sessions. To check on the generality of the findings from the original habituation training phase (Fig. 1), Fig. 6 shows responding in the reassigned groups averaged over the final two sessions. As before, RI-3 s and RI-6 s produced both a decline in response rate and a clear variety effect. A schedule (2) by reinforcer type (2) by 1-min bin (30) ANOVA found a main effect of schedule, $F(1, 20) = 14.84$, $MSE = 2133.29$, $p = .001$, and reinforcer type, $F(1, 20) = 7.85$, $p = .01$, but no schedule by reinforcer type interaction, $F(1, 20) < 1$. There was also a main effect of bin, $F(29, 580) = 21.58$, $MSE = 57.09$, $p < .001$, suggesting reinforcer habituation, but no interactions with the bin factor, $F_s < 1$. Thus, a variety effect was produced for the first time in these groups when we increased the reinforcement rate.

Figure 7 shows response rates in the new RI-3 s and RI-6 s Mix and Sucrose groups plotted as a function of 10-reinforcer blocks. The results in the new groups were entirely consistent with those observed in the original training phase. A reinforcer type (2) by schedule (2) by block (6) ANOVA found no main effects of schedule, $F(1, 20) < 1$, reinforcer type, $F(1, 20) = 1.76$, $p = .19$, or a schedule by reinforcer type interaction, $F(1, 20) < 1$. However, there was a significant main effect of block, $F(1, 20) = 23.89$, $MSE = 47.55$, $p < .001$. Although

block did not interact with schedule, $F(1, 20) = 4.25, p = .053$, it did interact with reinforcer type, $F(1, 20) = 7.66, p = .012$. As in groups initially trained with RI-3 s and RI-6 s, a variety effect emerged with both of these schedules as pellets were earned. The three-way interaction was not significant, $F < 1$.

Prefeeding test

Figure 8 shows responses over 6-min bins for different reinforcers and schedules for prefed and non-prefed rats in the final satiation test. (The figure plots 6-min bins instead of 1-min bins to compensate for the fewer data points in this test.) Prefeeding hastened the decline in responding evident during the test, but its impact was similar in all groups. Thus, variety did not increase food-seeking's resistance to the effects of satiation. A prefeeding (2) $\times$ schedule (2) $\times$ reinforcer (2) $\times$ bin (5) ANOVA revealed significant effects of schedule (RI-3 s vs. RI-6 s), $F(1, 40) = 44.06, MSE = 8990.97, p < .001$, pre-session feeding, $F(1, 40) = 19.99, p < .001$, and reinforcer type, $F(1, 40) = 12.76, p = .001$. There was also a significant interaction between schedule and feeding, $F(1, 40) = 8.41, p = .006$. Prefeeding seemed to produce a larger decrement in groups trained on RI-6 s, yet this may have been due to greater overall responding in these groups. There were no other between-group interactions, $F_s < 1$. Responding decreased as a function of the 6-min bins, $F(4, 160) = 98.89, MSE = 1868.43, p < .001$. The decrease interacted with reinforcement schedule, $F(4, 160) = 3.76, p < .01$, and prefeeding, $F(4, 160) = 8.45, p < .001$. Additionally, there was a marginal three-way bin $\times$ prefeeding $\times$ reinforcer interaction, $F(4, 160) = 2.24, p = .067$, suggesting that, while prefeeding caused responding to decrease more rapidly across the session, the variety effect remained intact. Other interactions were not significant (largest $F = 1.12$). To investigate the variety effect, responding in prefed animals was further analyzed. A schedule (2) $\times$ reinforcer (2) $\times$ bin (5) ANOVA confirmed a significant variety effect, $F(1, 20) = 6.71, MSE = 7648.76, p = .02$, as well as greater responding in RI 6-s, $F(1, 20) = 8.21, p = .01$. Variety and schedule did not interact in prefed groups, $F < 1$. Responding decreased as a function of 6-min bins, $F(4, 80) = 77.94, MSE = 1793.09, p < .001$. The decrease interacted with variety, $F(4, 80) = 3.10, p = .02$, suggesting that the variety effect decreased across 6-min bins. Other interactions were not significant (largest $F = 2.37, ps > .06$).

Figure 9 shows response rates over 10-reinforcer blocks for prefed and non-prefed rats in the final satiation test. An interval (2) by reinforcer (2) by prefeeding (2) by block (6) ANOVA found a significant main effect of variety, $F(1, 40) = 9.16, MSE = 1185.36, p = .004$, but no other between-group effects (largest $F = 1.74$). The block effect was reliable, $F(5, 200) = 11.76, MSE = 61.33, p < .001$, as was the schedule-by-block interaction, $F(5, 200) = 3.09, p = .01$. However, there were no other interactions between reinforcer type, prefeeding, or block (largest $F = 1.89$). Pre-session feeding did not influence response rate when assessed as a function of blocks of 10 reinforcers. It also did not eliminate the variety effect.

Discussion

The present results further link the variety effect reported by Bouton et al. (2013), to the effects of food variety on food habituation. First, they indicate that the tendency for the rat's response rate to decline within the 30-min session increased as the inter-pellet interval

decreased (and reinforcement rate increased) over groups. This pattern remained evident when response rates were examined over blocks of reinforcers (instead of time), which controlled for possible differences in the rate at which the gut was filled (for example). The results thus link the declining response rate to food habituation rather than simple satiation. Second, the variety effect was also observed when the inter-pellet interval produced habituation (RI-3 s and RI-6 s). The exception may be the RI-12 s condition, where a barely discernible (though statistically-significant) decline in responding was observed without a significant variety effect. It is perhaps notable that there was a small trend toward a variety effect in the early portion of the session in that condition. What is most important to note is that the results clearly associate the variety effect with the most rapid rates of pellet presentation. Overall, the results suggest that the present variety effect thus depends on the same very short inter-pellet intervals that also support food habituation.

The further tests of the RI-12 s and RI-24 s groups also provided important information. First, a variety effect was not observed in these groups in an extended, 120-min session. Although response rates did decline in that session, responding in the groups given the sucrose/grain mix declined at the same rate as that in the sucrose-only groups. Whether the decline in responding was due to habituation, satiation, or fatigue cannot be determined. However, the result clearly suggests that high rates of reinforcement, and not merely a declining rate of behavior, are necessary for observing the variety effect. Second, the RI-12 s and RI-24 s groups quickly demonstrated variety and habituation effects when the inter-pellet intervals were decreased to RI-3 s and RI-6 s. This suggests that within-session habituation and variety effects can be a rapid consequence of introducing low inter-pellet intervals. The pattern is consistent with evidence of rapid increases in responding on high rate schedules after a switch from a consistent food type to a mixture of two pellet types within session (Bouton et al., 2013).

Interestingly, the variety effect seemed to take different forms in the RI-3 s and RI-6 s groups. Rats responded at a high rate for the mixture of grain and sucrose pellets on RI-3 s at the beginning of the session and then converged with the sucrose group toward the end of the session. In contrast, the mixture and sucrose-only groups on RI-6 s responded at similar rates at the beginning of the session, but diverged later in the session. It is notable that this difference in pattern was not reliable when we examined response rate as a function of reinforcers rather than time (Fig. 2), where the interaction between block, schedule, reinforcer type fell far short of statistical significance. Additionally, the difference was not found when the RI-12 s and RI-24 s rats were switched to RI-3 s and RI-6 s (Fig. 6), but this may have been due to smaller sample size when analyzing data from only two sessions. The overall pattern might tentatively suggest that a consequence of receiving the mixture of pellet types at the very rapid 3-s interpellet interval (RI-3 s) could have been the enhancement, or sensitization, of responding during early portions of the session. It is noteworthy, however, that we found little direct evidence of a sensitization of responding (defined as an increase in responding over time) when we looked for it in the early-session data.

Other results suggest that the mixture of sucrose with grain pellets caused a variety effect without causing an increase in the pellets' reinforcing value. First, the mixed groups given

RI-3 s or RI-6 s did not sustain a higher rate of responding when the pellets were discontinued in extinction. Second, pre-session feeding did not change the variety effect. Persistence in behavior despite pre-session feeding has been taken as a measure of behavioral momentum (e.g., Nevin & Grace, 2000). The fact that the mixed-pellet groups showed no more resistance to the effect of prefeeding than the sucrose-only groups suggests that the mixture did not actually change the momentum of behavior. Thus, both the extinction and prefeeding tests suggest that the mix had no longer-term effects on response strength. Instead, its effects were restricted to those moments in which the pellets were earned at relatively high rates.

In contrast to food variety, reinforcement schedule had an impact on both the extinction and resistance-to-prefeeding measures of behavioral persistence. In extinction, responding decreased more slowly after RI-6 s than RI-3 s training, consistent with the partial-reinforcement extinction effect in simple schedules of RI reinforcement (e.g., Cohen, 1998). A similar pattern was observed after pre-session feeding, where the RI-6 s animals sustained their performance more than the RI-3 s animals. Thus, in addition to its modulation of the habituation and variety effects, inter-pellet interval also controlled differences in the long-term persistence of the operant food-seeking response.

It is well established that habituation of instrumental responding depends on short inter-pellet intervals (Lupfer-Johnson et al., 2010; McSweeney, 1992; McSweeney et al., 1996). The present experiment provides the first evidence, however, that the variety effect produced by different food types is similarly dependent on short inter-food intervals. Previous animal studies have not linked the variety effect as clearly to habituation because they did not show that habituation (or short inter-food intervals) was necessary to produce the effect (Lupfer-Johnson et al., 2010), did not provide evidence that the foods added to make the mixture were not merely more reinforcing than the reference food (Melville, Rue, Rybiski, & Weatherly, 1997), or were ambiguous in the sense that they found greater responding for the mixture of pellet types prior to the development of demonstrable habituation (Bouton et al., 2013).

Epstein et al. (2009) discussed a theoretical approach to food habituation that was based on Wagner's (1981) SOP model of learning and conditioning. In their framework, within-meal eating is subject to habituation as it is understood by SOP. Briefly, when a stimulus is contacted, a memory node corresponding to that stimulus is put in a high state of activity (the A1 state), which then decays rapidly to a lower level of activity (the A2 state). From there, the node decays more slowly and ultimately becomes inactive again (the I state). The A1 and A2 states are analogous to focal and peripheral processing of an item in memory, respectively. The sequence of activation states is unidirectional, always moving from A1 to A2 to I. Activation cannot move from A2 to A1. Epstein et al. (2009) suggested that when a food item (e.g., a sucrose pellet) is delivered, a memory node for sucrose is briefly activated to the A1 state, which we assume controls consumption behavior (including lever pressing here). The memory node then rapidly decays to A2, which temporarily makes it impossible for the node to return to A1. If sucrose presentations occur in rapid succession, habituation will occur because activation of the sucrose node to the A1 state can only be accomplished when it is allowed to decay to the I state. However, when foods are intermixed, consuming a

new food item (e.g., a grain pellet) will activate a memory node for *grain*. Because the number of nodes that can be in the A1 state simultaneously is limited (i.e., short-term memory capacity is limited), grain presentation will tend to restore the memory node for sucrose to the I state, ready to be activated back to A1. In addition, the functional interval between successive sucrose presentations doubles. By either mechanism, responding to the next sucrose pellet after consumption of a grain pellet would be less habituated.

The perspective clearly predicts that inter-pellet interval will affect both habituation and the variety effect. The shorter the interval between stimulus presentations, the more likely the second presentation will occur when the memory node is still in the A2 state, reducing its ability to enter A1 and control responding. Longer inter-pellet intervals allow the node to decay from A2 to the I state, providing greater opportunity for the next presentation to go to A1, and increase responding. Consistent with these predictions, the current RI-3 s and RI-6 s schedules arranged short inter-pellet intervals that allowed both habituation and variety effects, whereas the longer RI-12 s and RI-24 s intervals provided little evidence of them. At the theoretical level, the results thus imply that the sucrose node returned from A2 to I somewhere between 6 and 12 s after the last pellet presentation. In practical terms, the results suggest that eating in a way that keeps the last sensory experience fresh in mind (i.e., still in A2) is likely to enhance habituation compared to eating in a way that allows the information to decay or be replaced by new information. The fact that distraction (e.g., provided by television watching; Temple, Giacomelli, Kent, Roemmich, & Epstein, 2007) can increase eating and decrease habituation is also consistent with these conclusions.

An alternative theoretical approach is sensory-specific satiety (Rolls, 1986), which describes the finding that organisms presented with the same food repeatedly show a reduction in their hedonic reaction (e.g., pleasantness rating) to that food. The reduction in the hedonic reaction to repeated food could reduce motivation to work for food, resulting in lower response rates with a single food than a mixture of foods (Hetherington, Foster, Newman, Anderson, & Norton, 2006; Rolls & Hetherington, 1989). It is further possible that increasing the interval between food presentations would reduce memory for the pleasantness of the last food, resulting in less reduction, or no reduction, in responding for food presented with long inter-stimulus intervals. However, we are not aware of any systematic investigation of the effects of inter-stimulus interval on the development of sensory-specific satiety. In contrast, the role of inter-stimulus interval is explicitly recognized by habituation theory, which in any case may provide a mechanism that underlies sensory-specific satiety (e.g., Epstein et al., 2009; Hetherington et al., 2006).

Overall, the results suggest that the present example of the variety effect is specific to situations in which the food pellets are delivered at a high rate. Variety did not appear to enhance food's reinforcing value when it was assessed in terms of behavioral persistence (either extinction or resistance to satiation). The most straightforward explanation is that the present example of the variety effect is due to variety's short-term impact on food habituation.

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Highlights

- Variety increased food-seeking when short inter-food intervals produced habituation.
- Long inter-food intervals did not support habituation or the variety effect.
- Variety did not increase long-term behavioral persistence.
- The effect of food variety was due to its influence on short-term food habituation.

Fig. 1.

Mean lever press rates of the groups during each minute of the final six sessions. Group Mix = grain and sucrose pellets unpredictably; Group Suc = sucrose pellets.

Fig. 2.

Mean lever press rates of the groups during each successive block of ten reinforcers. Group Mix = grain and sucrose pellets unpredictably; Group Suc = sucrose pellets.

Fig. 3.

Mean lever press rates of the RI-3 s and RI-6 s groups during each minute (left column) and average session response rates in the final training and extinction session (right column).

Error bars represent *SEM*.

Fig. 4.

Mean lever press rates during the extended session. Groups RI-12 s (top panel) and RI-24 s (bottom panel) average response rates in 4-min bins. Group Mix = grain and sucrose pellets unpredictably; Group Suc = sucrose pellets.

Fig. 5. Mean lever press rates of Groups RI-12 s and RI-24 s during each successive block of ten reinforcers in the extended session. Group Mix = grain and sucrose pellets unpredictably; Group Suc = sucrose pellets.

Fig. 6.

Mean lever press rates of the RI-12 s and RI-24 s groups during each minute after a switch to RI-3 s and RI-6 s schedules. Group Mix = grain and sucrose pellets unpredictably; Group Suc = sucrose pellets.

Fig. 7. Mean lever press rates during each successive block of 10 reinforcers in two sessions following a switch from RI-12 s and RI-24 s to RI-3 s and RI-6 s schedules. Group Mix = grain and sucrose pellets unpredictably; Group Suc = sucrose pellets.

Fig. 8. Pre-session feeding test responding. Mean lever press rates of the RI-3 s (left column) and RI-6 s groups (right column; includes switched rats) during each minute of a non-prefed (top row) and prefed session (bottom row). Group Mix = grain and sucrose pellets unpredictably; Group Suc = sucrose pellets.

Fig. 9. Pre-session feeding test responding. Mean lever press rates of the RI-3 s (top) and RI-6 s groups (bottom) during each successive block of 10 reinforcers. Group Mix = grain and sucrose pellets unpredictably; Group Suc = sucrose pellets.