Incentive Contrast as a Relative Reward

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Incentive Contrast as a Relative Reward Process:

Using sucrose solutions in a single session to

test rapid reward comparisons in rats

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Abstract

This study examines the relationship between rapid relative reward comparisons and incentive contrast among rats ($n=5$). Animals were trained to lever-press in order to obtain access to a sucrose solution (concentration used: 1%, 10% or 20% in tap water). These rewards were placed outside an operant box which could be reached through a small hole displaying sessions with mixed comparisons (1v20%, 20v1%) or single sessions (1v1%, 10v10%, 20v20%) that rotated between two spouts containing the pre-randomized order of paired blocks; allowing for comparative analysis between two spouts/concentrations and blocks of responses. Throughout weekly testing each animal experienced a value upshift (positive) or downshifts (negative) relative to another outcome as we examined the incentive contrast effects on behavioral performance. We examined the influence of dynamic comparisons between the two reward outcomes in a repeated measures design with three sessions: a single outcome and a mixed outcome followed by a single outcome session the next day for extinction. Results signified rats experienced negative contrast and scaled their behavioral responses in decreased motivated action to obtain the incentive reward. Positive induction, however, was not obtained and proposes further research and analysis to understand the comparative values and to determine when motivational systems are registered to initiate behavior in animal paradigms. The future direction of this novel design and research area could be essential for investigating interactions between external and internal factors of motivation and reward processing as learning continues to play a role in conditioning and predictive contrast.

Keywords: Incentive contrast, relative reward, motivation, reward processing
1. Introduction

The study being introduced investigates models of motivation and mental illness in terms of addictive behaviors rising from current research objectives mirrored in the Affective Behavior and Motivation lab at Bowling Green State University. This project examines incentive contrast as a relative reward. Incentive contrast effects can be explained in the form of behavioral responses after an increase in reward (positive contrast) or decrease (negative contrast) (Binkley, Webber, Powers, Cromwell, 2014). As relative reward value has been extensively studied throughout comparative psychology using food rewards in animal models, behavioral paradigms have supported the idea that animals perform these relative reward judgments automatically and effectively by scaling their behavior proportional to relative shifts in reward value (Binkley et al., 2014). It has been well established that animals such as rats who typically receive a high valued outcome as an incentive will then show a negative response to a value downshift, negative contrast (Papini, Pellegrini, 2006; Papini, Seal, Pellegrini, 2008). As found in recent work done in this lab, results showed that animals express a greater relative reward effect when there is a larger difference between the reward outcomes (Webber, Chambers, Kostek, Mankin, Cromwell, 2015).

This study looks to fundamentally measure relative reward in relation to literature concerning the ‘Crespi Effect’ (Crespi, 1942); which discovered how the size of the reward has little to no influence on speed of learning but is most important when examining influence on tasks already learned. As a basis, reward is not defined in quantity but quality of last incentive learned, this means that a rat will respond to a task at the rate of its expected reward, which is in correspondence to the last reward received. The experiment examines this effect by measuring the response rate in relation to the previous reward received.
Weber’s Law also helps explain the rationale for this experiment as it looks at the relationship between magnitude of reward and intensity of stimulus. Weber’s Law states the observed size of the difference threshold appears to be directly related to initial magnitude of stimulus (Papini & Pellegrini, 2006). This can also be seen in other studies by Pellegrini and Papini, who shaped an experiment resulting in behavioral responses measuring lever-press quantity of rats to be similar in groups experiencing a 32→8% or 16→4% downshift in sucrose concentrations (post-shift to pre-shift ratio of 0.25) as well as another group experiencing a downshift of 32→4% or 16→2% (ratio of 0.125) relative reward (Pellegrini & Papini, 2007). The anticipatory ratio in these experiments were directly proportional to the response quantity in lever-press such that the 0.125 conditioned performance during the downshift phase was much lower (depressed) than the 0.25 ratio groups. Here the experiments follow Weber’s law indicating that behavioral change was determined by the downshift ratio and not the full magnitude involved in the depression (Pellegrini et al., 2008); also seen in consummatory response experiment examining appetitive behaviors in which rats drank varied sucrose concentrations when the incentive values were downshifted in 0.125 and 0.25 ratios (Papini & Pellegrini, 2006).

Successive negative contrast (SNC) is also a widely studied field of research that helps address the rationale for research questions concerning this study. SNC is often measured in two types, instrumental successive negative contrast (iSNC) and consummatory successive negative contrast (cSNC) (Papini & Pellegrini, 2006). Rats have the ability to learn and locate rewards that are preferred due to quality and quantity. Rising from such experience, a downshift in incentive value typically leads to more quantifiable depression of behavior than a control condition which receives less desirable rewards in all trials (Daniel, Wood, Pellegrini, Norris,
Papini, 2008); this is referred to as iSNC when it occurs due to anticipatory instrumental situations and cSNC when it occurs due to direct contact with the reward itself. However, since iSNC has not been seen when using sucrose concentration as the downshifted incentive, cSNC can be obtained in terms of licking responses in operant goal directed boxes (Flaherty & Caprio, 1976). Due to previous literature obtaining successful cSNC with such behavioral measures, we look to continue using similar measures of behavior in our study as well as examine positive contrast.

By using magnitude of food pellets to determine this effect in the previous study, no consummatory behaviors were able to be observed (see Webber et al., 2015). In the current study, these consummatory behaviors can be examined by using food in the form of sucrose concentrations just as Papini and Pelligrini (2006) have done in previous studies. Consummatory behaviors can be observed more clearly with varying sucrose concentrations due to the possibility of examining lick rate as a behavioral measure in relation to motivated states. Comparisons in this study use incentives varying in magnitude in the form of sucrose solution so that high magnitude can be achieved without inducing satiety which can be seen when excessive amount of food pellets are consumed (Flaherty et al., 1973). With the use of sucrose concentrations, ratio of magnitude reward can be increased by heightening the concentration or lowering the concentration instead of manipulating the number of pellets (Webber et al., 2015) which could lead to potential satiation effects across animals, disrupting true behavioral responses. To produce incentive contrast amongst testing sessions, sucrose concentrations will be varied similar to previous experiments such that 1%, 10% and 20% concentrations will be used (see Flaherty et al., 1973). Since relative hedonic value of sucrose solutions has been noted to influence the anticipated contrast value between outcome variations (Flaherty et al., 1973) we
can examine the adjacent behavioral response according to latency to lever press, consumption between trials and other consummatory measures (Pellegrini & Papini, 2007).

The experiment plans to deal with potential ceiling effects by introducing a variable delay interval between trial shifts. The VI (variable interval) will cause inhibition of the behavioral response associated with lever pressing in order to minimize learning effects and ceiling effects associated with learned responses. Seen in the Mellgren experiments involving delay periods and incentive contrast, he found success in using delayed periods to separate trials because the excitation due to reward expectancy was counteracted by an inhibitory factor produced by the delayed reward (Mellgren, 1972). Thus, by introducing a delay interval, ceiling effects can be avoided and therefore can provide a way to more clearly associate the behavioral responses with the reward expectancy (incentive contrast) and not other factors.

The objectives of this study are to more clearly understand the biopsychology of animal behavioral paradigms of learning, motivation and neural basis of reward. Since this study is an additive measure of another experiment involving relative reward and incentive contrast using food pellets in rats, this study aims to examine the same objectives while using different incentives (sucrose concentrations), which also means examining different types of behavioral measurements (Webber et al., 2015). These manipulations of variables and interval delay give us the ability to explore expectancies and the relationship between conditioned stimuli and anticipation on motivation as well as learning.

2. Methods

2.1 Animals

Five (n =5) Sprague-Dawley male rats (ages 8 months -18 months old) were used in this
experiment. Animals were housed individually (65 x 24 x 15 cm cages) and ranged in weight from around 350-575 grams at the beginning of testing sessions. The Animal Facilities Office at Bowling Green State University carried out all animal general husbandry procedures. Animals were quarantined for 3 days before regular housing procedures and habituated to a colony room for one week after their quarantine period. The colony room was on an automatic 12:12 hour light: dark cycle beginning at 8am (temperature 22°Celsius with 40%-50% humidity). All procedures had approval from the Institutional Animal Care and Use Committee at Bowling Green State University before the start of the experiment.

2.2 Food restriction and equipment

Rats were given a time period to get acclimated with the colony room in which they were given ad libitum access to food and water. Before testing animals were food deprived at target levels of 87-90% of their baseline weight. Animals were given 5-15 grams of food each day following testing to maintain the deprived weight so that consistent behavioral performance can be measured across multiple testing days.

Behavioral chambers (31 x 31 x 25cm) are used for training the animal to perform a lever press task for sucrose solution (10%). The operant chamber is connected to a computer using the software MED-PC (Med. Associates Inc. VT) to run custom programs written so that the operant box can accurately collect all related data.

2.3 Sucrose Concentrations

Three sucrose concentrations were mixed using distilled water and sucrose (sugar). A 1% sucrose concentration involving mixing 5 grams of sucrose with 495mL of distilled water. A 10% concentration with 50 grams of sucrose and 450mL of distilled water, along with a 20% sucrose
concentration with 100 grams of sucrose and 400mL of distilled water. The beakers of solution were made prior to testing and placed in a cold fridge where they remained. The solutions were only removed from the fridge during the testing period. During testing, 10mL of solution from the beaker was taken and placed in a clear spout and connected to the main apparatus located on the outside the operant box. The apparatus holds two spouts on both ends while the middle holding position is empty throughout testing (see Figure 1). The middle spout is empty to provide the delay period where no sucrose concentration is accessible for consuming and to test behavioral latencies to lever press as a means of achieving the sucrose reward.

![Figure 1. Apparatus, spins on the axis with attached spouts of sucrose solution. Connected to wires leading to inputs and outputs that direct the program and collect data. Located on the outside of the operant box but spouts are accessible through a small hole on the side of the box when spun to certain positions. The position of the spout is read through sensors located on the bottom of the attached spout holders (circular piece).](image)

2.4 Behavioral training

Animals were trained to lever press for three types of pellet flavors on a fixed ratio schedule of reinforcement (FR-1). After the rats pressed 30 times per 30-minute session, they were able to advance to sucrose training. Rats were trained to enter the operant chamber and lever-press after a FR interval while being exposed to a sucrose concentration of 10% for a 40 second period. Sucrose training finished after 4 sessions of 30 minutes in order for the animals to learn to lever-press and search for the sucrose reward in the correct location.

2.5 Behavioral testing

During behavioral testing animals were exposed to different blocks of reward outcomes across the days of testing. Each session consisted of 10 blocks of trials, each trial contains a shifted outcome block of either low concentration (L) to high concentration (H), high to low (HL) or maintained LL and HH controls. Low (L) concentration refers to 1% sucrose while...
high (H) concentration refers to 20% sucrose solution. The first day of each week over the period of two weeks only runs one single session of 10% sucrose concentrations in each spout (two spouts) in order to acclimate the rats to the operant chamber following a three day break from the weekend, where testing did not occur. The rest of the testing days exposed each rat to two sessions per day with 20-minute break periods in between sessions. Each session ends after 20 lever-presses (2 per block) and lasts around 15-20 minutes. The session begins after the rat enters the operant box, a lever then extends after a variable interval (VI) time period and once pressed will expose a sucrose spout for 20 seconds of free licking while a house light remains on. Once the period of free licking expires, the spout will move back into an empty position while the house light turns off and another VI is randomly selected to occur. The VI can be anywhere from 10-30 seconds (10, 15, 20, 25, 30) with an average selection of 20 seconds. After this period, the lever extends again and once pressed will expose the other sucrose spout for another 20 seconds. This continues to shift between spouts and the VI for 10 block trials with days consisting of two spouts of solutions filled with either HH (20v20% sucrose), LL (1v1% sucrose), LH (1v20% sucrose) or HL (20v1%) session pairings. The shifted combinations between testing days can be seen in Table 1.0. Ordering for sucrose outcome testing will be counterbalanced between subjects.

### 2.6 Statistical Procedures

The experimental analysis will run within-subjects, repeated measure procedures using SPSS software to analyze data. Main factors will be session block and sucrose concentration for

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<td>1) 1v1</td>
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<td>2) 20v20</td>
<td>2) 20v1</td>
<td>2) 1v1</td>
<td>2) 1v20</td>
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<td>2) 20v1</td>
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the analysis. Separate analyses will be done on these main factors to explore incentive contrast (session block for the same outcome) and discrimination (different sucrose concentrations). Pair comparisons for the different blocks (1 vs. 2 or 2 vs. 3 or 1 vs. 3) and for the different sucrose concentrations (1 vs. 10 or 10 vs. 20 or 1 vs. 20% sucrose) will be completed to determine differences between sets of outcomes. Wilcoxon signed-ranks test will be used as a non-parametric alternative to a t-test that assumes rank differences between paired groups, blocks in this case (single sessions and mixed sessions; LL, HH, and LH, HL) because there is no means to make assumptions about the shape of distribution. The test essentially calculates the difference between each set of pairs and analyzes these differences in rank order, which were used to make comparisons within specific magnitude combination session types.

2.7 Hypotheses

The results of this lab are expected to provide us with a larger relative reward effect as a behavioral expression of incentive contrast than seen in our previous studies (Webber et al., 2015). Since this experiment will be using different incentives, sucrose solutions instead of sugar pellets, as a relative reward, the experiment is predicted to increase the effect of consummatory behaviors by avoiding satiation and connecting the behavioral responses directly to contrast of reward values. Since our experiment excludes any issues related to satiety our relative reward effect is expected to be larger than in previous studies (Webber et al., 2015) due to our quantifiable value magnitude of varying concentrations of sucrose. We anticipate a strong negative contrast for the lower concentration when paired with the higher during session series that begin with the lower concentration solution (1 vs. 20%) and positive contrast for the higher concentration solution when the initial session is the higher concentration solution (20 vs. 1% sucrose solution).
3. Results

**Negative Contrast Session:** To test negative contrast we compared 10 trials of lever-press responses anticipating 1% solution in the single session (1% vs. 1%) to responses anticipating 1% sucrose in the mixed session (1% vs. 20%). Negative contrast was obtained (see Figure 1A) as responses were significantly slower in the mixed session compared to the single session ($W = 15.0; p = 0.043$). Response discrimination between the LH concentrations were analyzed by comparing the 1% versus the 20% in the mixed session. Findings did not indicate significance but showed a trend ($W = 14.0; p = 0.080$) signaling slower response time in the 20% compared to the 1% (see Figure 2A).

**Positive Contrast Session:** To test positive contrast, we compared 10 trials of lever-press responses anticipating 20% solution in the single session (20% vs. 20%) to responses anticipating 20% solution in the mixed session ($W = 9.0; p = 0.686$) and to responses anticipating 1% solution in the mixed session ($W = 2.0; p = 0.138$). These results did not provide significant findings to showcase evidence of positive contrast in the single versus mixed sessions and neither in the discriminated HL mixed sessions (see Figure 2B).

**Figure 2.** Shows negative contrast of the session types across the entirety of testing (Figure 2A). The negative contrast can be clearly observed in the single and mixed sessions of average latency to lever press within the 1v1 single session and the 1% solution discriminated from the mixed LH. Positive contrast is analyzed as well in Figure 2B but is not defined in either the discriminated mixed sessions of 20% and 1% following the single session of 20v20%.
Session Statistics: Throughout testing we recorded basic statistics including the total volume consumed across all animals (n=5) in 2 sessions (through two 10mL spouts), mean session time in seconds ± SEM (the session will automatically close after 25 minutes, 1500 seconds), mean trials completed ± SEM (40 possible, 2 sessions, each 20 trials), and the mean volume calculated by consumption individually (n=5) per day (2 sessions), see Table 2.0. Monday was used as an acclimation period where each rat experienced one session of 10% single solution in each 10mL spout, which included 15 lever-presses to solution before closing the session. The same statistics were taken for week 2 testing and can be seen in Table 3.0. The weights were also recorded each day across the two-week testing period and can be seen in Figure 3.

Figure 3. The weights across the two weeks stay consistently above critical weights (85% of baseline) and rest around target weights (87% baseline) but do slowly decrease throughout the weekly sessions due to deprivation. 2-4 food pellets were given to each animal after each testing day to maintain weight. Mean weight of all the animals combined is included as well for each week, labeled average.

Table 2.0. Session statistics from Week 1 (7/14/15 - 7/17/15).

<table>
<thead>
<tr>
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<th>Tuesday</th>
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<tr>
<td>Total Volume</td>
<td>7.35</td>
<td>9</td>
<td>8.5</td>
<td>15.7</td>
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<tr>
<td>Consumed</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Across all</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals (per day)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean Session</td>
<td>881.506 ± 72.0</td>
<td>1019.062 ± 104.15</td>
<td>1187.152 ± 110.19</td>
<td>1141.447 ± 106.35</td>
</tr>
<tr>
<td>Time ± SEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Trials</td>
<td>39.6 ± 0.40</td>
<td>36 ± 4.0</td>
<td>27.4 ± 6.06</td>
<td>27.6 ± 7.83</td>
</tr>
<tr>
<td>Completed ± SEM (40 possible)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Volume</td>
<td>1.47 ± 0.251</td>
<td>1.8 ± 0.5205</td>
<td>1.7 ± 0.705</td>
<td>3.14 ± 0.720</td>
</tr>
<tr>
<td>Consumed Individually per Day ± SEM</td>
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Table 3.0. Session statistics from Week 2 (7/21/15 - 7/24/15).

<table>
<thead>
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<th>Week 2</th>
<th>Tuesday</th>
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<tr>
<td></td>
<td>20v1 Mix</td>
<td>20v20 Single</td>
<td>1v20 Mix</td>
<td>1v1 Single</td>
</tr>
<tr>
<td>Total Volume Consumed Across all Animals (per day)</td>
<td>24.2</td>
<td>25.1</td>
<td>15.2</td>
<td>21.6</td>
</tr>
<tr>
<td>Mean Session Time ± SEM</td>
<td>744.772 ± 22.03</td>
<td>690.102 ± 13.92</td>
<td>811.17 ± 79.17</td>
<td>969.818 ± 118.37</td>
</tr>
<tr>
<td>Mean Trials Completed ± SEM (40 possible)</td>
<td>40 ± 0</td>
<td>40 ± 0</td>
<td>38.4 ± 1.60</td>
<td>34.8 ± 4.49</td>
</tr>
<tr>
<td>Mean Volume Consumed Individually per Day ± SEM</td>
<td>4.82 ± 1.072</td>
<td>5.02 ± 0.472</td>
<td>3.04 ± 0.826</td>
<td>4.34 ± 1.206</td>
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</table>

Single Session Statistics: Single session statistics were recorded and compared to see if the average latencies across the single concentration days were significantly different. Paired samples t-tests revealed that none of the single concentration latencies were significant (1v10%, 10v20%, 20v1% single days) but when comparing 20% to 1% single concentration days, \( p = .079 \), marginal significance was calculated. See Figure 4.

Figure 4. Average latencies for the single session days (of week 2) were recorded by taking 5 random trial latencies from each of the two sessions (10 random trials total) on each day the single concentration was administered. Standard error is also recorded.

4. Discussion

The results showed significant findings when examining negative contrast sessions, indicating that the animals scaled their behavioral responses in juxtaposition to the decrease in relative reward of sucrose concentration. This means that the rodents performed at a decreased level of urgency when lever-pressing for a negatively contrasted reward of 1% as opposed to the
high valued reward of 20% sucrose concentration following a 1% vs. 1% session. From these findings we can clearly observe the decreased motivation to achieve the low valued reward as our hypothesis predicted. However, the same positive contrast or positive induction was not seen in this group of rodents as hypothesized when the high valued 20% concentration was simultaneously compared to the 1% concentration following a 20% vs. 20% session.

Our expectancy of positive contrast incentive value was not achieved and may be due to the low sample size of animals. The animals were combined from different cohorts who have had different previous experiences in a lab setting. Animals 1-3 (7A, 8A, 10A) were all from the same cohort while 4-5 (11C, 14C) originated from a different cohort. When examining just animals 1-3 and their behavioral responses corresponding to average latencies, positive contrast was found. This could be due to differences in cohort response patterns as animals 4-5 followed the similar patterns but were in opposition to the response patterns of animals 1-3. These pattern results provided insignificant findings among response latencies due to the pull of two separate patterns of response variation. In the future we may choose to use animals from the same cohort to control for differences in experience and responding patterns.

Another potential explanation for discussion on why this study and other studies have failed to find positive contrast within their behavioral measurements is the predictability of the relative reward comparison (Webber et al., 2015). Since the reward comparison is dynamic in this experiment, the distinction of where and when the animals are choosing to determine this relative reward response pattern are questionable. We are unable to clearly understand if the distinction is occurring as a generalization of the experimental session or within the blocked trials of negative and positively contrasted sucrose solutions. This reward comparison could also potentially occur at unpredictable rates either before or after the switched solution which would
impact how the response data was analyzed. If the animals were responding to the incentive in context to the previous reward received as other work has noted (Crespi, 1942; Flaherty, 1973), the analysis of that trial would occur before they received the next trial as a predictive set. However, the argument could be made that the generalization and predictive quality of each trial following the other could be learned and behavioral responses would be analyzed in context to that understanding. Either way, this idea needs to be further investigated in future studies to determine when this relative reward comparison is occurring.

A potential issue that may result in inconclusive positive contrast findings could arise from ceiling effects. The variable interval was introduced in this study to potentially abolish any ceiling effects (Mellgren, 1972) causing a delay in the appetitive task aimed at ceasing quick automatic responses. As seen in response times across the weeks, findings in the 20v20% sessions occurred at such a fast rate that ceiling effects are brought into question. Similar learning effects may have occurred across weeks as responses times decreased in the second week and consumption increased. To counteract the ceiling effects and other reward comparison issues, introduction of tone cues could be added to future experiments, which could help clear uncertainty with prediction and response rates. Learning effects in the future could also be examined with more sessions of weekly contrasts. However, this could raise satiation concerns and may suggest positive induction instead of solving learning effects across the weeks.

In addition, the study hoped to explore connections between affective state and relative reward comparison by monitoring rat ultrasonic vocalizations (USVs). The basis of this idea stems from previous research stating that affective state influences incentive contrast with rodents using distinct sounds to signal internal emotion (Webber et al., 2012). Dissociations between behavioral and USV indicators of contrast have been found (Binkley et al., 2014)
supporting the idea that affective state changes occur but need more clarification if they translate into behavioral paradigms (Webber et al., 2015). For example, 50 kHz USVs are very sensitive to social reward systems (Webber et al., 2012) which can be seen as emitting high sensitivities when anticipatory incentive value is also high (Binkley et al., 2014). The sucrose reward used along with moderate food restriction may induce sufficient motivation to lead to outcome modulated USV signals as not seen in previous work done in this lab. In the end this additional analysis was unable to be performed and acquired due to shortage of inputs available in our unit program. In the future, we would hope to include this aspect of affective states in order to investigate these connections further.

Exploring reward comparison is an important area of research within experimental psychology involving behavioral neuroscience and animal models of motivation and learning (Webber et al., 2015). The modified approach of this experiment incorporating sucrose solutions for dynamic comparison processes lead to the influential findings of negative contrast and continued search of positive contrast in behavioral paradigms. The future direction of this design and research area could be essential for investigating interactions between external and internal factors of motivation and reward processing as learning continues to play a role in conditioning and predictive contrast.
References


reward effects on operant behavior: Incentive contrast, induction and variety effects.

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