

**REWARD DEVALUATION IN A TWO-LINK CHAIN SCHEDULE:  
EFFECTS OF REWARD DENSITY IN THE PROXIMAL LINK AND  
FOOD RESTRICTION PROTOCOL IN RATS**

by

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## Abstract

Food restriction has been shown to affect responding for reward and has been manipulated to devalue reward. Reward density has been shown to alter responding in both first-order schedules and chain schedules. Devaluation has differential effects in first-order schedules and the links of chain schedules. The objective of the current study was to analyze the reward devaluation effect in a two-link chain schedule by manipulating food restriction and reward density in the proximal link; these variables had previously not been studied systematically. The chain schedule required rats to lever press according to a random interval 120-s schedule to turn on a light conditioned stimulus (CS). In the presence of the CS rats were rewarded for pulling a chain; the chain was active on a variable ratio (VR) 5 schedule of reinforcement for some rats, while the chain was on continuous reinforcement (CRF) for the other rats. Food restriction protocols were either 1-hr daily free feeding (1-hr feeding) or a daily ration to maintain weight at 80% of free feed controls (ration). Devaluing the CS while in the isolated proximal link was done by exposing trained rats to sessions of chain pulling in the presence of the CS but withholding reward. In subsequent lever press sessions without the CS, decreased responding was seen in rats that had undergone the devaluation procedure, but only in rats on the 1-hr feeding protocol. This devaluation effect was found in both the CRF and VR 5 schedules. Subsequent tests of lever pressing for the CS also revealed decreased responding. Post-test CS discrimination sessions demonstrated CS devaluation that has not been demonstrated previously. These results demonstrate that the isolated devaluation of the proximal link in a heterogeneous two-link chain schedule can result in decreased responding in the isolated first link. This effect is

dependent on sufficient food restriction and demonstrates a measurable devaluation effect which is independent of the reward being present. This study also demonstrated that isolated devaluation of the second link can be subsequently measured in a post-test CS discrimination re-acquisition session regardless of food restriction, which had no significant effect in the session. The devaluation effect has not been demonstrated in a re-acquisition session before, rather responding in previous re-acquisition sessions were only affected by food restriction.

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## List of Abbreviations

CRF – Continuous Reinforcement

CR – Conditioned Reward

CS - Conditioned Stimulus

FI – Fixed Interval

FR – Fixed Ratio

RI – Random Interval

RT – Random Time

VI – Variable Interval

VR – Variable Ratio



## Chapter 1: Introduction

The underlying elements of animal behavior have fascinated people for centuries. The current study examined how animal behavior is affected by reward. An outcome is defined as rewarding if an animal is willing to work to obtain it. The effects of food restriction on responding for food reward are generally that the stronger the food restriction, the stronger the responding. In contrast, food reward can be devalued in a number of ways, including allowing consumption of the food reward in a satiated state and stimuli associated with reward can be devalued, for example by withholding the reward after an operant response. Prior exposure to a food reward in a specific motivational state can affect subsequent state-specific responding for that reward, suggesting that the value of a reward is learned. Animals can be trained to perform multiple types of responses in heterogeneous chain schedules, with the responses proximal to the reward being more affected by the current motivational state, while responses more distal to the reward were more affected by the learned value of the reward.

Incentive learning involves the acquisition by neutral stimuli of the ability to elicit approach and other responses to reward-related stimuli. These responses can be measured in a variety of ways, including, but not limited to conditioned place preference, conditioned activity to pro-dopaminergic drugs, and instrumental responding for reward. Instrumental responding is commonly measured by key pecking in pigeons, or wheel turning, chain pulling, and lever pressing in rats on various reinforcement schedules. Schedules can be linked together to produce chain schedules where the completion of one link in the schedule provides a cue and allows access to the next link in the schedule.

The behavior elicited by the individual links when combined in a chain schedule is fundamentally different than behavior elicited by the same schedules administered alone, as explained below (Corbit & Balleine, 2003).

The term *reward* can be dissociated into *wanting* and *liking*. Wanting is described as being willing to work for reward; that is what is measured in instrumental responding. Liking can be described as the affective, emotional state of pleasure as a result of reward. Thus, wanting is not the subjective enjoyment of a reward, while liking does not mean the animal is willing to work for the reward, no matter how pleasurable the experience may be. In the current paper, *reward* is used in the same meaning as wanting. Thus, reward is the driving force behind incentive learning. For reviews of the liking-wanting dissociation see Kelley and Berridge (2002), Berridge (2003), Berridge & Robinson (2003).

The current study was performed to measure the devaluation effect on distal lever press responses when proximal chain pull responses have an isolated history of non-reward. In a 2 X 2 design, the effects of food restriction protocol (1-hr feeding or ration) and the number of proximal responses necessary to obtain reward (one or an average of five) were assessed on devaluation.

## Chapter 2: Literature Review

### *Food Restriction*

Food restriction manipulations affect motivational state and have been shown to alter the rate of magazine training acquisition, lever press acquisition, and final lever press rate (Lattal & Williams, 1997; Dickinson, Smith & Merenowicz, 2000). Food restricted animals that were pre-fed normal chow before testing demonstrated increases in the interval between lever press bouts on a variable interval (VI) schedule (Shull, 2004). Mild fluctuations of food restriction intensity have been shown to affect within-session responding on a multiple schedule (Pinkston, Saulsgiver & Branch, 2007). Thus, food restriction protocols can influence experimental outcomes and have been manipulated in some reward devaluation experiments (see below).

Food restriction has been directly manipulated in experiments of incentive learning, demonstrating that it is not only the motivational state during testing that is important, but also the motivational state in which the food reward was previously experienced. Balleine (1992) pre-exposed some rats to food reward while others were not pre-exposed prior to all rats undergoing instrumental training and then testing in extinction. In addition, food restriction in each phase of the experiment (pre-exposure, training, testing) was manipulated. It was found that rats pre-exposed to food reward while food restricted, trained while satiated, and then tested while food restricted demonstrated increased responding compared to rats that were not pre-exposed, or rats that were pre-exposed in a satiated state. This increase in responding was found only when rats were tested in extinction while food restricted. Interestingly, rats pre-exposed

to the food reward while satiated, trained while food restricted, and tested while satiated, performed the operant less than animals that were not pre-exposed to the food reward or animals that were pre-exposed while food restricted.

In sum, when rats were pre-exposed to a food reward in the same motivational state as they were tested in extinction, then there is evidence for stronger incentive learning compared to non pre-exposed rats, or rats tested in a different motivational state. It is a learned effect, not a performance effect because the pre-exposure manipulation occurred days prior to testing. Differences attributed to *learning* would be expected when the experimental manipulations occur *prior* to testing, while differences attributed to *performance* effects would be expected when experimental manipulations occur *during* testing. It is possible for learning to occur within a test session, evidenced as a change in behavior within a session.

In the same experiments by Balleine (1992) it was found that it was not motivational state during extinction, per se, that dictated responding for reward, but rather congruency of motivational state and previous incentive learning. Contrary to what may be expected, it was found that rats tested while food restricted did not respond for reward more than rats tested while satiated, *unless* they were pre-exposed to the food reward in the same motivational state as testing. If the differences were only due to performance effects, then motivational state during testing alone would predict responding during testing. Since the behavior measured in testing depended on pre-exposure and food restriction manipulations completed days prior to testing, it is a learned effect.

It is important that Balleine's (1992) experiments tested in extinction as testing in extinction did not expose the rats to the food reward itself to be re-evaluated. Re-acquisition rate of the operant response in a rewarded re-acquisition session depended on current motivational state, with no significant effect of incentive learning found. This is not surprising, as the rats were re-exposed to the reinforcer during the re-acquisition session, and the food reward was re-evaluated according to the current motivational state and the incentive value of the food reward was adjusted accordingly. Since the re-evaluation may occur rapidly, it may be difficult to measure without appropriate temporal resolution. Magazine entries were measured during testing by how often rats pushed a hanging panel out of the way to gain access to the food magazine. Interestingly, magazine entries depended on motivational state during testing, but not pre-exposure, a finding that was not fully appreciated until after the completion of follow-up experiments from the same lab, explained below in the section on chain schedules.

In summary, food restriction can act as a powerful tool in manipulating incentive learning. It has previously been demonstrated that food restriction results in a motivational state that determines the incentive value of food reward encountered in that motivational state through a process of re-evaluation. This incentive value is maintained until re-evaluation of the food reward occurs through re-exposure. If an animal is acquiring, maintaining, or re-acquiring instrumental responding for reward, then the reward is constantly re-evaluated. This constant re-evaluation is dependent on the motivational state of exposure to the reward or reward-related stimuli. The same constant re-evaluation in a stable motivational state and stable outcome results in stable

responding. There are methods which use re-evaluation to change the incentive value of reward.

### *Reward Devaluation*

Reward devaluation is the decrease in incentive value of reward by a process of re-evaluation. Food reward devaluation can occur in a variety of ways including feeding of the food reward itself while the animal is satiated (Balleine, 1992; Dickinson, Balleine, Watt, Gonzales, & Boakes, 1995), pre-feeding of other foods (Dickinson et al., 2000, Shull, 2004), pre-reward administration of dopamine receptor antagonists such as haloperidol (Salamone, 1986) or pimozide (Wise, Spindler, deWit, Gerber, 1978; Pecina, Berridge, & Parker, 1997; Dickinson et al.; Johnston et al., 2001), post-reward administration of lithium chloride (Holland, 2004; Igushi & Ishii, 2006), and simply withholding of reward following the operant response (Salamone; Johnston et al.; Shull & Grimes, 2006; Duarte, Biala, Le Bihan, Hamon, & Thiebot, 2003; Wise et al.). Each method decreases operant responding, but through different mechanisms and there are measurable differences in the characteristics of the operant responding from each method (e.g., Salamone, Johnston et al., Dickinson et al., Pecina et al.).

The differential effects of the aforementioned reward devaluation procedures can be partially explained by acknowledging a difference between *wanting* and *liking*. The extinction-like decrease that dopamine antagonists have on responding for reward was initially interpreted as ‘anhedonic’ (Wise et al., 1978), but subsequent research has been able to demonstrate that dopamine antagonists selectively reduce responding for reward (wanting), but do *not* change measurements of hedonic liking, such as orofacial

movements and paw licking (Pecina et al., 1999). Since the current study does not measure liking, the term *reward* refers only to wanting in the current paper.

There is also a dissociation between devaluation of primary reward and devaluation of conditioned reward (CR). For instance, the malaise induced by post-reward administration of lithium chloride works well if the reward is the food reward itself (Holland, 2004), but is less effective when the reward is a CR (Igushi & Ishii, 2006; for a review see Di Chiara, 2002). Manipulations such as motivational state are believed to devalue food reward itself, as do the lithium chloride injections. Other manipulations are less direct, such as withholding reward, or administering dopamine receptor antagonists, that seem to devalue the wanting of the reward. It is also possible to devalue discriminative stimuli when they act as CR, as described below.

In summary, there are many methods which result in decreased responding for reward, but they are not necessarily acting in similar ways to achieve the same result. These methods all work to decrease *wanting*, defined as working for an outcome, not the liking of the reward, which is the subjective feeling. There are studies which have devalued the primary reward itself, other studies which have devalued the outcome of responding, and studies which devalue stimuli associated with responding. These differences in methods all decrease responding for reward, but in different ways. It is the differences in these methods which become very useful when they are selectively used in devaluation studies utilizing chain schedules.

### *Chain Schedules*

Chain schedules are the combination of two or more first order schedules, called *links*, with distinct exteroceptive stimuli signaling each link. The stimuli may be brief or may be continuous during the entire link, such as the conditioned stimuli (CS) used in the current experiments. Brief stimuli increase responding compared to continuous stimuli or no stimuli (Thomas & Stubbs, 1967; Malagodi, DeWeese & Johnston, 1973). The link closest to the primary reward is referred to as being *proximal* while links farther from the primary reward are referred to as being *distal*.

Homogeneous chain schedules require responding on the *same* manipulandum for all links. Heterogeneous chain schedules require responding on *different* manipulanda for each link. For example, D'Andrea (1969) had rats respond on a vertical bar press active on a VI 60-s schedule for the distal link of the chain schedule. Completing the distal link turned on a CS and a horizontal bar became active on a variable ratio (VR) 10 schedule. Johnston et al. (2001) used a procedure similar to that used in the current experiments; it consisted of a VI 120-s lever press for a light CS. In the presence of the CS, a chain was active on continuous reinforcement (CRF) that turned off the CS and presented food reward. The CS for the chain schedule acts as a CR, with the strength dependent on the number of rewards presented during the CS (see Kelleher & Gollub, 1962 for a review).

A distinction can be made between reward devaluation in first-order schedules and chain schedules. In an example of a first-order schedule, there may be an action-outcome contingency learned between the operant response (action) and the food reward (outcome). When an outcome changes, it is re-evaluated upon the experience of the



outcome. That is to say, a lever press can be reinforced by the outcome being a pellet by the *experience* of consuming the pellet. When the action predicts the outcome in a stable manner, then stable actions are found. When an action no longer predicts the rewarding outcome, then there is a gradual drop in the action that occurs. The decrease is dependent on the number of action-no-outcome events, as one would expect if the outcome was re-evaluated based on the experience of the outcome, be the outcome rewarding or not rewarding.

In a schedule involving a discriminative stimulus, it is the action during the stimulus that is contingent on the outcome. Thus the action in and of itself cannot be devalued, as the action in and of itself does not predict reward. It is the discriminative stimulus that is devalued, as it no longer predicts reward. This is measured as a decrease in responding in the presence of the CS, as responding in the absence of the CS has no (or low) value. This can occur when the action is allowed during the CS, but no reward is presented. Thus, it is the experience of responding in the presence of the CS resulting in no reward that is subject to devaluation, not the experience of responding itself that is subject to devaluation.

In a two-link heterogeneous chain schedule, responses in the distal link presents a CS, which then acts as a discriminative stimulus for responding in the proximal link. If the proximal link is isolated from the distal link and the CS is presented but the reward withheld, then the CS is devalued, as responding on the proximal manipulandum in and of itself is of no value. Since the CS maintains responding in the distal link, it can be viewed as a CR. Thus, if the proximal link is isolated, and the CS devalued, then

responding in the distal link should decrease due to lower value of the CR. If the distal link is tested in isolation and the CS is not presented, then the responding will be for the *expected* value of the CS (as a CR). By not presenting the CS, it is not subject to re-evaluation as a CR. This is a crucial detail that allows full appreciation of devaluation protocols using isolated links of a heterogeneous chain schedule. A CS is devalued in an extinction session by re-evaluation (through experience) of the CS (action-outcome) contingency value. In the absence of the CS, *thus preventing re-evaluation of the CS as a CR*, responding for the CS is measured on the distal link manipulandum. If a decrease in responding is found, then it is due to an action-CS (action-outcome) expectancy that has been devalued, not simply the action-outcome contingency, or the stimulus (action-outcome) contingency. In sum, the chain schedule allows measurement of devaluation of a CS outcome that is not subject to an effect on the action-CS contingency, as the CS is not presented.

One manner of understanding this subtlety is to understand the current value of the CS is always interoceptive. In the case that the CS is present, the value is experienced concurrently exteroceptively as well as interoceptively, but when the CS is absent, the value can only be interoceptive. Direct re-evaluation is dependent on exteroceptive stimuli. If the stimuli are not exteroceptive, and thus not subject to direct re-evaluation, then a measured devaluation effect in their absence cannot be due to direct, exteroceptive re-evaluation processes. In the absence of the CS, there may be indirect, interoceptive re-evaluation processes. This is what is measured in heterogeneous chain schedules that devalue the proximal link in isolation from the distal link and measure a

devaluation effect on the distal link in the absence of the CS. This indirect, interoceptive re-evaluation process may occur in other devaluation protocols, but it is confounded by the exteroceptive presentation of the CS that is subject to direct re-evaluation. Thus the term *devaluation* refers to both the exteroceptive and interoceptive experience of the CS when the CS is present, but only to the interoceptive experience when the CS is absent.

Responding is higher in the distal link of a homogeneous chain schedule than the same response used as the distal link of a heterogeneous chain schedule (Lattal & Crawford-Godbey, 1985), that could possibly be due to the average temporal proximity of reward to the operant response, or the distal link having a stronger association with the proximal link because the response is the same. Increasing reinforcer rate in the proximal link of a two-link homogeneous chain schedule increases responding in each link (Davison & McCarthy, 1989). Work by Neuringer & Schneider (1968) suggests that it is the inter-reinforcement time, not the number of inter-reinforcement responses that predicts instrumental responding. This work manipulated fixed ratio (FR) and fixed interval (FI) schedules using blackout periods to control for inter-reinforcement time or the number of inter-reinforcement responses.

In a series of experiments Balleine, Garner, Gonzalez, and Dickinson (1995) expanded upon the Balleine (1992) experiments, demonstrating that incentive learning also occurs when the animal is re-exposed to the food reward after instrumental training, similar to the pre-exposure incentive learning effects described earlier. Furthermore, it was demonstrated that the magazine panel was similar to a proximal link in a two-link heterogeneous chain schedule, with the lever press acting as the distal link. When rats

were trained to lever press in the distal link, and chain pull in the proximal link of a two-link chain schedule without a magazine panel, the responses on the chain were similar to magazine panel entries in a replication of Balleine (1992). This demonstrated that the magazine panel was acting similar to a proximal link in a two-link chain schedule. These experiments also demonstrated that the distal link was sensitive to incentive learning and devaluation, but not necessarily motivational state during extinction testing, whereas the opposite pattern was found in the proximal link of the chain schedule, with chain pulls or magazine panel entries sensitive to motivational state, but not incentive learning, during extinction testing. Similar to Balleine (1992), it was found that motivational state, not incentive learning, predicted the rate of re-acquisition for responding for reward.

A chain VI 60-s VR 10 heterogeneous chain schedule, quite similar to the chain RI 120-s VR 5 schedule in the current study and by Johnston et al. (2001) demonstrated that response-contingent presentation of the discriminative stimulus for the proximal link acted as a CR by increasing responding in both links while the stimulus was on for 30 s in a within-session extinction session (D'Andrea, 1969). Royalty, Williams, and Fantino (1987) showed that a discriminative stimulus acts as a CR, the effect of which degrades over stimulus onset delay, similar to primary reward. Similarly, stimuli associated with a shorter proximal link in a two-link heterogeneous chain schedule increases the value of those stimuli as a CR (O'Daly, Angulo, Gipson, & Fatino, 2006). In an early review of CR, Kelleher and Gollub (1962) concluded that the function of a stimulus as a discriminative stimulus was neither necessary nor sufficient by itself to act as a CR; it is the pairing of stimuli with reward that makes them CRs.

Olmstead, Lafond, Everitt, and Dickinson (2001) used the term *seeking* to refer to appropriate responses during the distal link on the distal manipulandum, and *taking* to refer to appropriate responses during the proximal link on the proximal manipulandum in a heterogeneous chain schedule. These terms will only be used to refer to the links in a heterogeneous chain schedule. Olmstead et al. demonstrated in a two-link heterogeneous chain schedule that cocaine seeking and sucrose seeking, measured as responses on the distal manipulandum in extinction, were goal-directed actions that were susceptible to reward devaluation by withholding reward in the proximal link of the chain schedule while the proximal link was isolated from the distal link. Using similar procedures, Johnston et al. (2001) demonstrated that sucrose seeking was decreased similarly by withholding reward in the proximal link or by pre-session administration of the D2-like receptor antagonist pimozide.

### *The Current Study*

The distal and proximal links of a two-link heterogeneous chain schedule can be viewed as reward *seeking* and *taking*, respectively. In the current study the seeking response will be lever pressing and the taking response chain pulling. The action of the lever press turned *on* the CS, and primary reward was presented for chain pulling in the presence of the CS. Thus, both links had distinct stimuli, actions, and outcomes. The CS can be devalued by withholding reward for responses on the chain. If this is seen to lead to decreased lever pressing in the subsequent tests, it suggests that the neural representation of the CS can influence the strength of lever pressing.

If the CS is not presented during the lever press test session, and decreased lever pressing is observed, then it would be indicative of a neural or cognitive representation of the altered value of the CS that is *not dependent* on re-exposure to the CS itself. That is to say, that if the CS is not presented during the lever press test, then the decreased lever pressing in the non-reinforced group may be due to the interoceptive *expectation* of the value of the CS, not the presence of the CS itself. Not presenting the CS would also prevent any possible direct exteroceptive re-evaluation effects on the CS itself. If the CS is presented during lever press testing, then a decrease in responding could be due to the value, or re-evaluation, of the CS itself.

The objective of the current project was to analyze the reward devaluation effect in a two-link chain schedule by manipulating food restriction and reward density in the proximal link; these variables have not been studied systematically. The chain schedule required rats to lever press according to a random interval (RI) 120-s schedule to turn on a light CS. In the presence of the CS rats were rewarded for pulling a chain; the chain was active on a VR 5 schedule of reinforcement for some rats, while the chain was on CRF for the other rats. Using the VR 5 schedule should increase chain pulls during the extinction phase, possibly increasing the devaluation effect.

Pilot studies and previous research indicated that the method of food restriction may be an important factor in this paradigm, so food restriction protocol was manipulated, using either a 1-hr daily free-feed protocol (1-hr feeding) or a daily ration to maintain weight at 80% of free-feed controls (ration). These manipulations yield a 2 X 2 between subjects design. What is devalued in devaluation experiments is the CS. This is

done by exposing trained rats to sessions of chain pulling in the presence of the CS but withholding reward. In subsequent lever press sessions, decreased responding is seen in rats that have undergone the devaluation procedure.

To assess the possibility that changes to test-day lever pressing reflected general locomotor activity differences, an inactive lever was also present during training and testing. Increased lever pressing on both levers would be indicative of a general locomotor effect in the operant chamber. If there was no change in inactive lever presses, then a change in pressing the active lever could not be attributed to general locomotor changes.

The CS may have been acting as a CR, so a second test-day used in the 1-hr feeding experiments investigated the possible CR effects of the CS on lever press. If the CS was acting as a CR, then it would increase lever pressing selectively on the active lever.

The post-test light discrimination probe sessions demonstrated the extent of CS devaluation. It should be noted that in contrast to the literature cited above, that reinstated responding on the entire chain schedule itself, and the post-test light discrimination probe session specifically measured responding in the isolated proximal link of the chain schedule. Since the light discrimination score was independent of baseline chain pull rate, it specifically measures the devaluation of the light as a CS.

It is hypothesized that the group that does not receive food for pulling during isolated proximal link training (non-reinforced group) will demonstrate decreased lever press rate in both test days, compared to the reinforced group. The non-reinforced group

was also expected to demonstrate a decreased light discrimination score, based on the post-test light discrimination probe session. It is hypothesized that the 1-hr feeding food restriction will increase overall responding compared to the ration food restriction as 1-hr feeding was the stronger food restriction. The VR 5 chain pull schedule is hypothesized to increase chain pulls during the extinction phase, compared to the CRF chain pull schedule, increasing the exposure to non-reinforcement. This increased exposure may increase the devaluation effect, as measured in test-day lever press rate. The VR 5 chain pull schedule may also decrease baseline lever press rate, compared to the CRF schedule.



## Chapter 3: Methods

### *Subjects*

Male Wistar rats (N = 68; Charles River, Canada) weighing 300-350 g at the beginning of the experiment were food restricted (see below). Potentially, if rats were housed in groups or pairs, then dominant rats would likely consume more food than submissive rats, interfering with the precision of the food restrictions used. For this reason, rats were singly housed in an environmentally controlled room with a 12-hr light/dark cycle with lights on at 19:00 hr. Water was freely available in the clear plastic (40 x 25 x 22 cm high) home cages with the floors lined with 4 cm of wood chip bedding (Beta Chip; Northeastern Products Corp., Warrensburg, NY). All experiments were conducted in accordance with the Canadian Council on Animal Care and Queen's University Animal Care Committee regulations.

### *Apparatus*

Training and testing took place in four operant chambers (26.5 x 22 x 20 cm high) inside larger wooden boxes with a fan for air circulation and to mask outside noise. The back and sides of the operant chamber were made of stainless steel, while the front and top were clear Plexiglas. The floor of the operant chamber was 3-mm diameter stainless steel rods spaced 11 mm apart, parallel to the side walls. The operant chambers were fitted with two retractable levers 3.5 cm wide on opposing side walls equidistant from the front and back of the chamber and 2.5 cm from the floor. The feeder magazine was centered on the back wall 2.5 cm above the floor. A retractable chain manipulandum fabricated from a standard sink plug chain, with 4 mm diameter beads, can be inserted into the

operant chamber through a hole in the top of the chamber. The chain manipulandum was equidistant from the side walls and 5 cm from the front of the operant chamber with the bottom of the chain approximately 4 cm from the floor. Two houselights wired in parallel were mounted 8 cm above the feeder magazine, each 6 cm from the side wall, and acted as the CS.

### *Procedure*

Four experiments were performed in a 2 x 2 (food restriction x reward density) design. In two experiments the rats were given a daily food ration to restrict their weight to 80% of a free-feeding control rat (ration). In the other two experiments the rats were given daily 1-hr free access to food in their home cage (1-hr feeding). All weights were measured at the start of the day and all rats were fed (LabDiet 5001, PMI Nutrition Intl, Brentwood, MO) 30-60 min after completion of daily sessions. Reward density was manipulated by altering the reward schedule in the proximal link. For one experiment from each food restriction protocol, reward was presented in the proximal link of the chain schedule according to a CRF schedule and in the other experiment of each food restriction protocol reward was presented on a VR 5 schedule. In the following the training conditions for one of the experiments involving training on CRF in the proximal link in the chain schedule will be described. This will be followed by a description of how training varied for each of the remaining experiments.

### Training

Rats were trained on a chain RI 120-s CRF schedule with lever presses being rewarded according to the RI schedule with the CS (house light). Chain pulls in the presence of the

CS were rewarded with 45 mg food pellets (Bioserv, Frenchtown, NJ) according to the CRF schedule. There were two retractable levers in the chambers during lever press training and testing sessions: one of the two levers was designated as the *active lever* for each rat. Responses on the other *inactive lever* had no programmed consequence and served as a measure of general locomotor activity. The active lever (right versus left) was counterbalanced within groups within experiments.

*Chain pull training.* In the first stage of training, the levers were not presented and the rats were reinforced on a CRF schedule for pulling the chain manipulandum in the presence of the CS. Each chain pull dispensed a food pellet, turned off the CS, and started a random time (RT) schedule for presentation of the CS on the next interval. After the RT had elapsed, the CS was presented until the next chain pull. Rats received a maximum of 20 pellets in the maximum 2-hr session. Since most sessions did not take the full 2 hrs to complete, some sessions that required less time to complete were given in succession so rats spent an average of approximately 90 min in the operant chambers during each training day. On the first day of training 6 sequential sessions incrementing the RT between each session from 0 to 2 to 5 to 15 to 30 to 60 s were given. The next day all rats were given RT 120-s sessions until they reached the light discrimination criterion (see below). A light discrimination score was calculated as follows with “rate” measured in average pulls per second in a session:

$$\text{Discrimination} = \frac{\text{(Chain pull rate with CS on)}}{\text{((Chain pull rate with CS off) + (Chain pull rate with CS on))}}$$

This formula yields a score that controls for baseline differences in overall chain pull rate, with no light discrimination yielding a score of .50 and perfect light discrimination yielding a score of 1.00. Rats received up to 3 consecutive RT 120-s chain pull training sessions per day, until their light discrimination score was greater than .90.

*Lever press training.* The day after rats achieved discrimination criterion, the levers were inserted into the chambers. One lever was active, while the other was inactive. The first response on the active lever (reward seeking) started a RI schedule and the first response meeting the RI contingency turned on the CS, activating the chain manipulandum that rewarded rats on a CRF schedule, similar to chain pull training, turning off the house light when a pellet was delivered. The lever press RI was increased over daily sessions from 0 to 2 to 5 to 15 to 30 to 60 to 120-s intervals. The first four lever press sessions were given sequentially on the first day of lever press training. The fifth and sixth sessions were given on the second day of lever press training. A total of 5 daily sessions were performed at the RI 120-s to obtain baseline lever press rates (lever presses per reward) for each rat. Rats received a maximum of 20 pellets in each 2-hr session.

*Extinction phase.* This phase was referred to as the extinction phase, even though control (reinforced) rats did not undergo extinction. Half (n=8) the animals were assigned to the non-reinforced group and the other half (n=8) were assigned to the reinforced group. The levers were retracted and the chain remained in the chamber. For the reinforced group, the CS was presented on a RT 120-s schedule identical to the chain

pull training sessions. The first chain pull in the presence of the CS dispensed a pellet and turned off the CS. This first response in the presence of the CS is called a *taking* response in the extinction phase. The non-reinforced group was on the same RT 120-s schedule of CS presentation but no reward was given for the first chain pull with the CS on. Instead, the CS remained on for 20 s and then turned off, starting the next interval. Sessions lasted until 20 pellets had been dispensed (reinforced) or 1 hr had elapsed (non-reinforced). Daily sessions were administered until every rat in the non-reinforced group responded on 5 or less intervals, with a minimum of at least 5 sessions. All reinforced rats were trained until the last non-reinforced rat reached criterion, but once a non-reinforced rat reached criterion, it was left in the home cage until the end of the extinction phase.

*Testing.* The day after the last extinction session, lever presses were measured in a 30-min test session. Both levers were presented and the number of presses on each lever were recorded in 5-min bins. The chain was not present, nor was the CS or reward presented. Each 5-min bin for each rat was divided by the average lever press rate for that same rat during the 5 days of RI 120-s training to control for baseline differences in lever press rate. These were the ‘standardized lever press’ values used for analysis.

*Changes between experiments.* Rats in the VR 5 experiments were given 2 preliminary chain pull sessions, during which the reinforcement schedule gradually increased from CRF to VR 5. The VR 5 schedule was programmed to select, with equal probability on each trial, a number 1 to 9 to correspond with the number of chain pulls necessary to dispense reward. Immediately after the preliminary chain-pull sessions, the

VR 5 rats responded in increasing RT schedules of the CS incrementing between each sequential session from 0 to 2 to 5 to 15 seconds the first day. The second day, the VR 5 rats were given on two sequential sessions, one at RT 30-s, the second at RT 60-s. It is important to note that regardless of experiment, during the extinction phase only one chain pull in the presence of the CS was sufficient to complete the trial.

In the experiment using the ration food restriction and CRF proximal link (CRF ration) and the experiment using the ration food restriction and VR 5 proximal link (VR 5 ration), there was only one test-day. In experiments using the 1-hr feeding food restriction (CRF 1-hr feeding and VR 5 1-hr feeding) the second test-day had the CS turn on for 5 seconds consistent with the RI 120-s lever press contingency, but no chain or reward was presented. Test-day 2 was designed to measure the possible effect of the CS as a conditioned reward.

The day after lever press testing, a chain-pull probe session was given for both 1-hr feeding experiments, as well as the VR 5 ration experiment. The probe session was identical to the RT 120-s chain pull sessions during training. The light discrimination score of each rat was measured, as an indication of CS chain pull devaluation.

#### *Data Analysis*

All analyses were performed using SPSS version 11.5. The experimental design allows a 2 X 2 (food restriction X reward density) analysis of data. Where applicable, the comparisons were 'within group', but between experiments. For instance, CRF 1-hr feeding and VR 5 1-hr feeding non-reinforced groups were compared. Similarly CRF ration and VR 5 ration were compared to CRF 1-hr feeding and VR 5 1-hr feeding,

respectively, using food restriction as the between-subjects variable. No post-hoc tests were necessary. When repeated measures analysis of variance (ANOVA) was performed, trend analysis (contrasts) was performed in every case. Neither sphericity of variance nor a significant main effect of the within-subjects variable are assumptions of running trend analysis (Glass & Hopkins, 1996).

Rats were assigned to experimental groups based on baseline lever press rate. Baseline lever press rates were the average number of lever presses per reward in the 5 daily sessions of lever press RI 120-s schedule. Groups were assigned to have equal baseline lever press rates based on a 5-day repeated measures analysis of variance ANOVA with prospective group as between subjects.

## Chapter 4: Results

One rat in the non-reinforced group in the CRF ration experiment was removed due to being an outlier (4 SEM above the mean) in test day lever pressing. One rat in the reinforced group and three rats in the non-reinforced group in the VR 5 ration experiment were removed due to test day equipment failure. One rat in the VR 5 ration experiment failed to discriminate the light to criteria after 14 RT 120-s sessions and was removed.

### *Lever Press Test Day 1*

Standardized lever presses were analyzed for the test days to reduce between-subject variability due to baseline differences in lever press rate. The standardized lever presses were calculated as the number of lever presses during testing divided by the average number of lever presses per reward during baseline lever press training.

#### CRF Ration

There was no significant difference between reinforced and non-reinforced groups ( $F(1, 13) = .46, p = .510$ ; See Figure 1). Lever pressing decreased across 5-min bins in a linear ( $F(1, 13) = 45.97, p < .001$ ), quadratic ( $F(1, 13) = 20.37, p = .001$ ), and cubic ( $F(1, 13) = 5.92, p = .030$ ) function. There was no time X group interaction (all contrast X group  $p > .146$ ). Inactive lever pressing did not differ between reinforced and non-reinforced groups ( $F(1, 13) = 1.18, p = .298$ )

#### VR 5 Ration

There was no significant difference between reinforced and non-reinforced groups ( $F(1, 9) = 2.65, p = .138$ ). Lever pressing decreased across 5-min bins in a linear ( $F(1, 9) = 35.16, p < .001$ ) function. There was no time X group interaction (all contrast X group  $p$



> .2.90). Inactive lever pressing did not differ between reinforced and non-reinforced groups ( $F(1, 9) = .35, p = .568$ )

#### CRF 1-hr Feeding

Experience with extinction in the proximal link of the chain schedule resulted in lower lever press rates than those seen in reinforced rats ( $F(1, 14) = 8.28, p = .012$ ). Lever pressing decreased across 5-min bins in a linear ( $F(1, 14) = 52.49, p < .001$ ), and quadratic ( $F(1, 14) = 11.11, p = .005$ ) function. There was no time X group interaction (all contrast X time  $p > .190$ ). Inactive lever pressing did not differ between reinforced and non-reinforced groups ( $F(1, 14) = .16, p = .692$ )

#### VR 5 1-hr Feeding

Experience with extinction in the proximal link of the chain schedule resulted in lower lever press rates than those seen in reinforced rats ( $F(1, 14) = 5.07, p = .041$ ). Lever pressing decreased across 5-min bins in a linear ( $F(1, 14) = 35.52, p < .001$ ), and quadratic ( $F(1, 14) = 5.04, p = .041$ ) function. There was no significant time X group interaction (all contrast X group  $p > .189$ ). Inactive lever pressing did not differ between reinforced and non-reinforced groups ( $F(1, 14) = 3.11, p = .100$ )

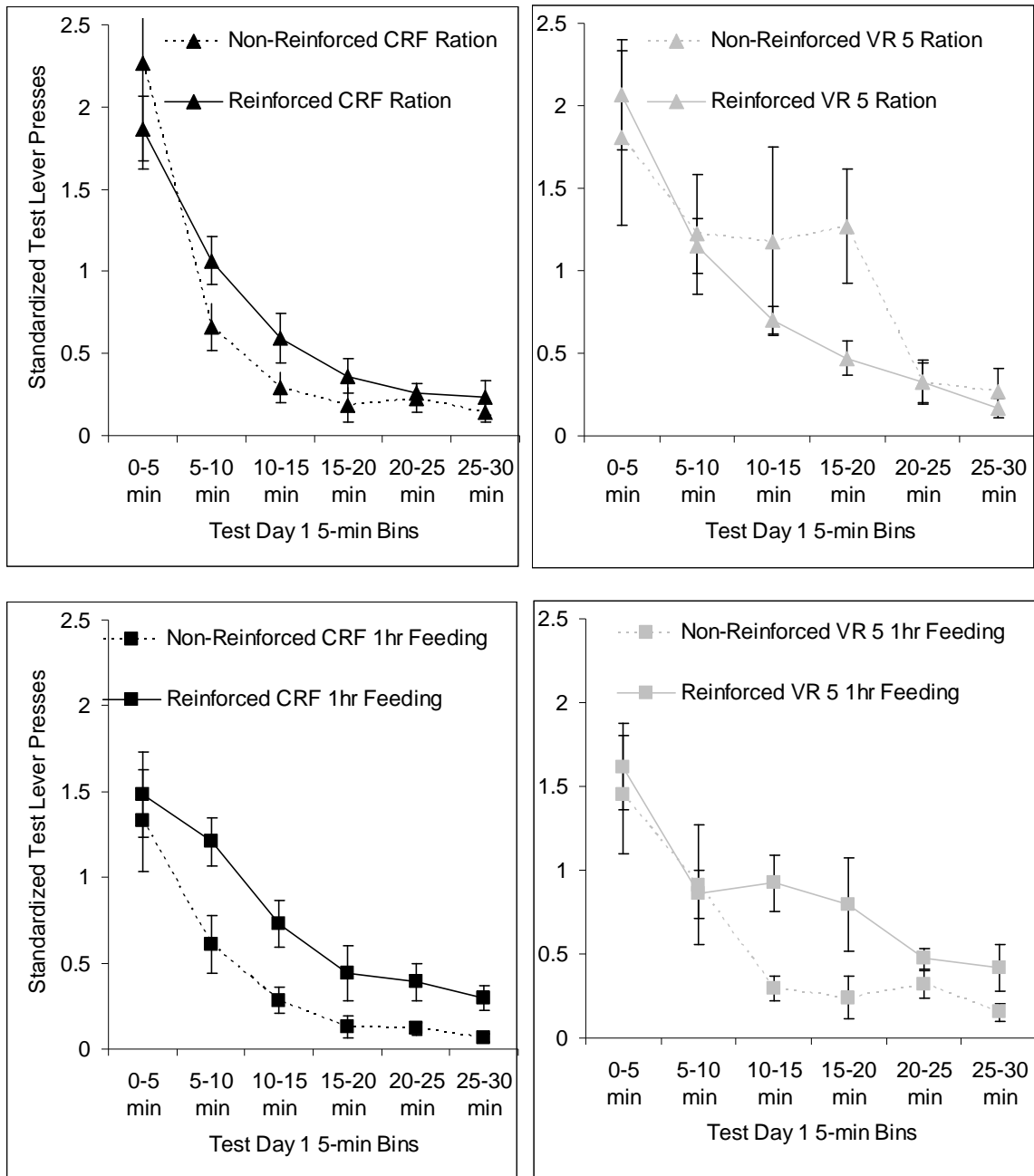


Figure 1: Test day 1 standardized lever presses.

Each experiment is displayed separately. Experiments using the CRF schedule in the proximal link are on the left, experiments using the VR 5 schedule in the proximal link are on the right. Experiments using the ration food restriction are on top, experiments using the 1-hr feeding experiments are on the bottom. Points represent group means, bars represent SEM. Both 1-hr feeding protocols resulted in significant devaluation effects, there was no significant devaluation effect in either ration experiment.

### *Lever Press Test Day 2*

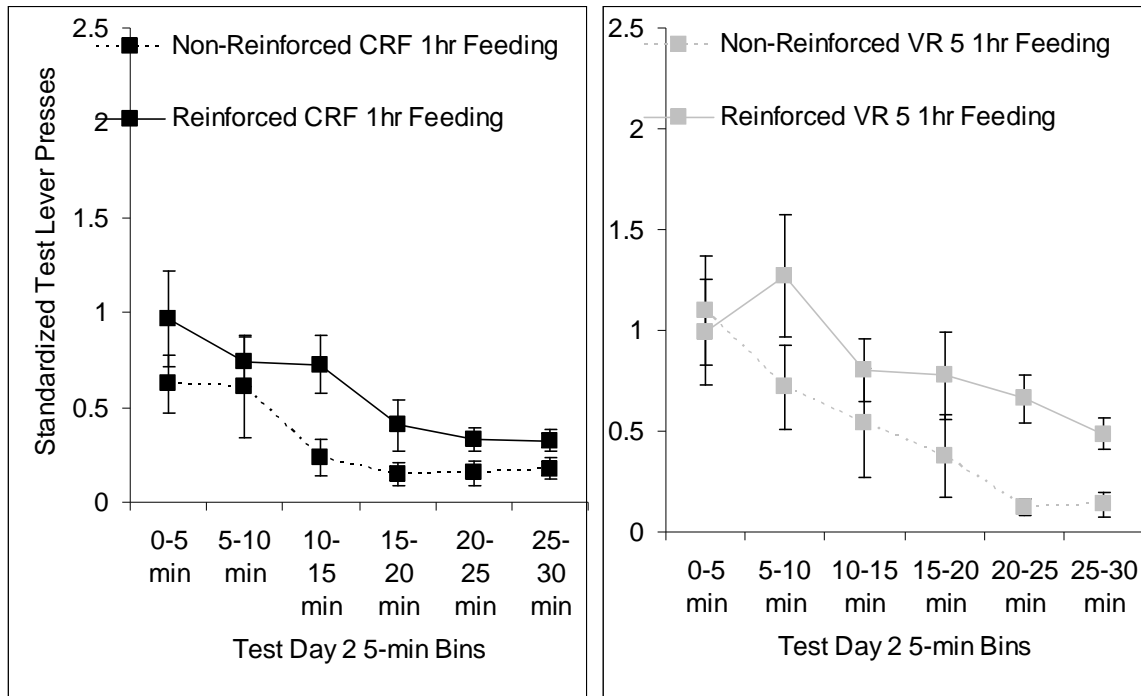
Lever press test day 2 was not administered in either ration food restriction experiment.

#### CRF 1-hr Feeding

Experience with extinction in the proximal link of the chain schedule resulted in lower lever press rates than those seen in reinforced rats, but the difference was not quite significant ( $F(1, 14) = 4.15, p = .061$ ; See Figure 2). Lever pressing decreased across 5-min bins in a linear ( $F(1, 14) = 22.33, p < .001$ ) function. There was no time X group interaction (all contrast X group  $p > .287$ ). Inactive lever pressing did not differ between reinforced and non-reinforced groups ( $F(1, 14) = .02, p = .901$ ).

#### VR 5 1-hr Feeding

Experience with extinction in the proximal link of the chain schedule resulted in lower lever press rates than those seen in reinforced rats ( $F(1, 14) = 7.59, p = .015$ ). Lever pressing decreased across 5-min bins in a linear ( $F(1, 14) = 20.24, p < .001$ ) function. There was no time X group interaction (all contrast X group  $p > .235$ ). Inactive lever pressing did not differ between reinforced and non-reinforced groups ( $F(1, 14) = 2.15, p = .165$ ).



*Figure 2: Test day 2 standardized lever presses.*

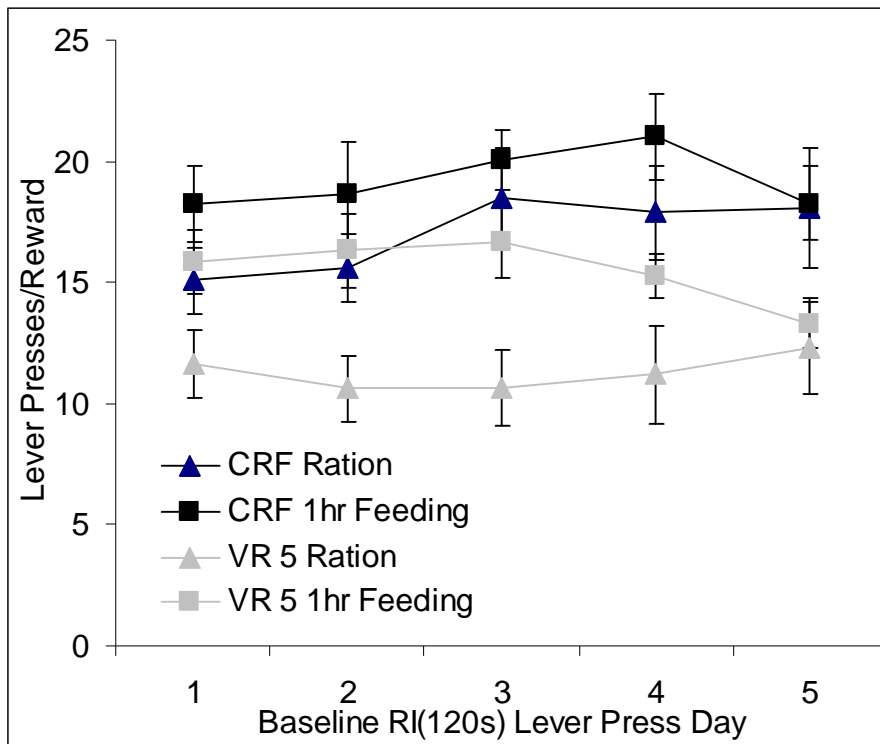
Each experiment is displayed separately. Experiments using the ration food restriction protocol did not receive test day 2; experiments using the 1-hr feeding experiments are displayed. Points represent group means, bars represent SEM. Both 1-hr feeding experiments resulted in devaluation effects, which was significant in the VR 5 experiment and near-significant in the CRF experiment.

#### *Baseline Lever Press Phase*

Rats on the 1-hr feeding protocol demonstrated more lever presses per reward than the ration protocol while rats on the CRF schedule in the proximal link demonstrated more lever presses per reward than the VR 5 schedule in the proximal link (See Figure 3). Rats in the 1-hr feeding protocol demonstrated a shallow inverted-U change in responses over days. This interpretation is supported by the results of a 2 X 2 X 5 (food restriction X schedule X day) ANOVA using lever presses per reward as the dependent variable.

There was a main effect of schedule ( $F(1, 54) = 11.37, p = .001$ ) and a main effect of

food restriction ( $F(1, 54) = 5.20, p = .027$ ). There was a suggestive quadratic trend over days ( $F(1, 54) = 3.70, p = .060$ ), and a day X food restriction interaction ( $F(1, 54) = 4.42, p = .040$ ). Follow-up repeated-measures contrasts over days indicated a quadratic trend in the 1-hr feeding experiments ( $F(1, 31) = 11.57, p = .002$ ) but not in the ration experiments ( $F(1, 25) = 0.02, p = .892$ ).



*Figure 3: Baseline lever press rates.*

Baseline lever press rates (lever presses per reward) are compared between experiments. Reinforced and non-reinforced groups were not yet assigned, so the data are from all rats within an experiment. The VR 5 schedule reduced lever press rate compared to the CRF schedule. The 1-hr feeding food restriction experiments demonstrated increased responding compared to the ration food restriction experiments. There was no food restriction X schedule interaction. Reinforced and non-reinforced rats did not differ on baseline lever press rate.

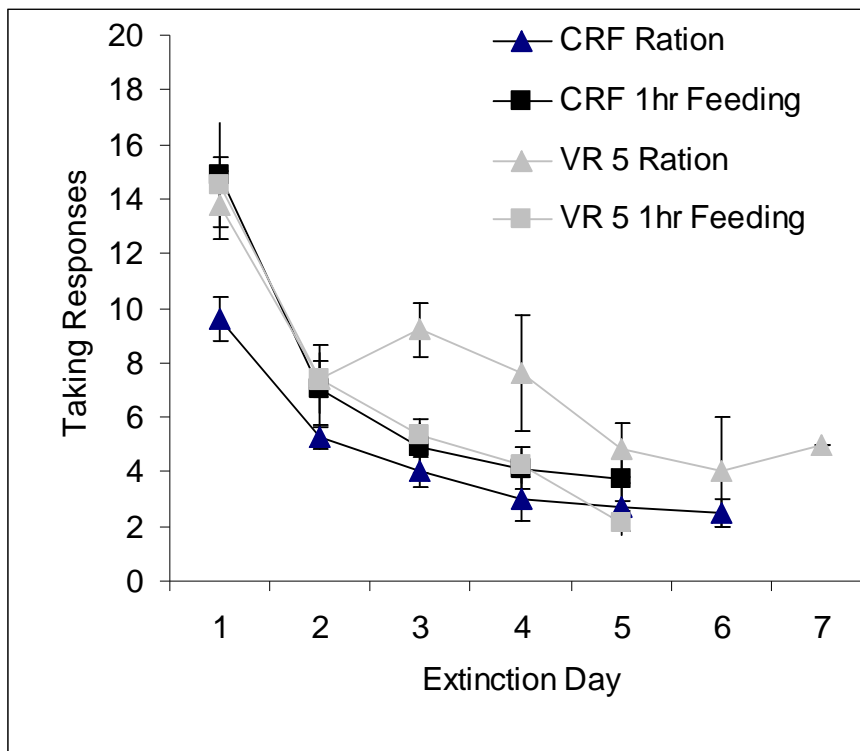
As rats were assigned to reinforced and non-reinforced groups based on baseline lever press rates, there were no significant difference in the reinforced and non-reinforced groups in any of the experiments (all  $p$ 's > .35).

#### *Extinction Phase*

All reinforced rats responded at the maximum amount of 20 pellets on every extinction day in all experiments. The non-reinforced groups in each experiment responded less than the reinforced rats (all  $p$ 's < .001, data not shown).

The non-reinforced rats reached extinction criterion after 5 (1-hr feeding VR 5, 1-hr feeding CRF), 5 or 6 (ration CRF), and 7 (ration VR 5) days training. Analyses were performed on the first 5 days of extinction to compare the effects of food restriction and reinforcement schedule on rate of extinction. Analyses collapsed across all days of extinction produced nearly identical statistical results; in all cases the conclusions following analyses are the same, so these analyses are not reported. Non-reinforced rats in each experiment demonstrated a linear-quadratic-cubic decrease in responding over days. Rats trained on the VR 5 schedule using the ration food restriction demonstrated the strongest resistance to extinction in the first 5 days (See figure 4). This interpretation is supported by the results of a 2 X 2 X 5 (food restriction X schedule X day) ANOVA using taking responses as the dependent variable. There was a main effect of schedule in the proximal link ( $F(1, 24) = 5.87, p = .023$ ) but no main effect of food restriction ( $F(1, 24) = .02, p = .903$ ) and a linear ( $F(1, 24) = 135.89, p < .001$ ), quadratic ( $F(1, 24) = 61.96, p < .001$ ), and cubic ( $F(1, 24) = 17.94, p < .001$ ) trend over days. There was a schedule X food restriction interaction ( $F(1, 24) = 7.32, p = .012$ ). Follow-up analyses

indicated a significant increase in responding in the VR 5 schedule compared to the CRF schedule in the ration experiments ( $F(1, 10) = 16.36, p = .002$ ) but not in the 1-hr feeding experiments ( $F(1, 14) = 0.04, p = .847$ ).



*Figure 4: Non-reinforced groups responding in the extinction phase.* Taking responses, defined as the first chain pull in the presence of the CS, during the extinction phase. Reinforced group responding was at the maximum of 20 responses per daily session (data not shown).

#### *Post-Test Probe Session*

##### Devaluation Effect

The post-test probe session was not administered in the CRF ration experiment. There was a decrease in light discrimination in the non-reinforced groups compared to the reinforced groups in the VR 5 ration ( $F(1, 9) = 14.48, p = .005$ ) and VR 5 1-hr feeding

( $F(1, 14) = 8.68, p = .011$ ) experiments. The same comparison in the CRF 1-hr feeding experiment approached significance ( $F(1, 14) = 4.27, p = .058$ ).

#### Reinforced Rats

There was no significant effect of food restriction on the VR 5 trained reinforced rats ( $F(1, 12) = 0.00, p = .993$ ) or an effect of reinforcement schedule on reinforced rats in the 1hr feeding food restriction ( $F(1, 14) = .032, p = .860$ ).

#### Non-reinforced Rats

There was no significant effect of food restriction on the VR 5 trained non-reinforced rats ( $F(1, 10) = 0.09, p = .774$ ) or an effect of reinforcement schedule on non-reinforced rats in the 1hr feeding food restriction ( $F(1, 14) = .834, p = .377$ ).

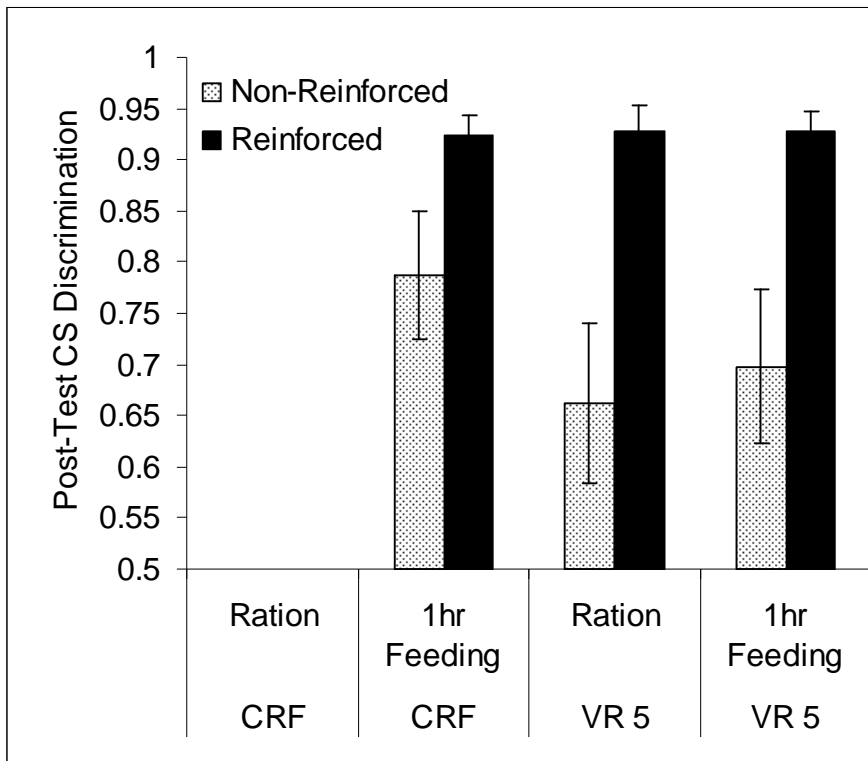


Figure 5: Post-test CS discrimination scores.

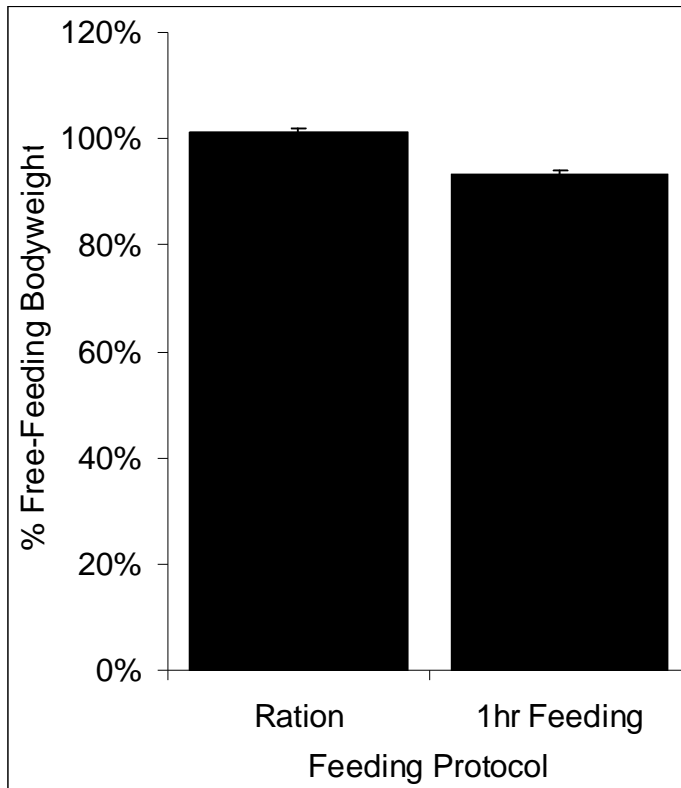


There was a significant CS devaluation effect in all studies in which the post-test CS discrimination session was administered. The post-test CS discrimination session was not administered in the ration CRF experiment. There was no significant difference between experiments for the non-reinforced groups nor was there a significant difference between experiments for the reinforced groups. See methods for calculation of the CS discrimination score.

#### *Percentage Free-feeding Bodyweight*

Free-feeding bodyweight was the weight of the rats the day they were switched from free-feeding to food restricted. Each rat was compared to their own free-feeding bodyweight to obtain a percentage free-feeding bodyweight. The average percentage free-feeding bodyweight was calculated using all days of the experiment. The rats in the CRF and VR 5 experiments on the ration food restriction protocol weighed more ( $M = 97.5\%$  and  $105.3\%$ , respectively) than the corresponding experiments on the 1-hr feeding protocol ( $M = 93.6\%$  and  $93.0\%$ , see figure 6). The 1-hr feeding experiments differed with respect to non-reinforced and reinforced groups ( $M = 95.1\%$  and  $91.5\%$ , respectively) whereas the ration protocol groups did not differ ( $M = 101.1\%$  and  $102.2\%$ ). This interpretation was supported by a 2 X 2 X 2 (food restriction X schedule X group) ANOVA using average bodyweight as the dependent variable. This resulted in a main effect of food restriction ( $F(1, 50) = 81.22, p < .001$ ), a main effect of schedule ( $F(1, 50) = 13.43, p = .001$ ), a food restriction X schedule interaction ( $F(1, 50) = 19.03, p < .001$ ), and a food restriction X group interaction ( $F(1, 50) = 6.20, p = .016$ ). There was no main effect of group, or any other interactions with group (All  $p$ 's  $> .190$ ). Simple effects analysis demonstrated that the ration food restriction protocol resulted in heavier weights than the 1-hr feeding protocol in the CRF experiments ( $F(1, 29) = 11.54, p = .007$ ) and VR 5 experiments ( $F(1, 25) = 69.18, p < .001$ ). The VR 5 rats weighed more

than the CRF rats in the ration experiments ( $F(1, 24) = 76.52, p < .001$ ) but not the 1-hr feeding experiments ( $F(1, 30) = 0.16, p = .690$ ). The reinforced rats weighed less than the extinction rats in the 1-hr feeding experiments ( $F(1, 30) = 5.77, p = .023$ ) but not the ration food restriction experiments ( $F(1, 24) = 0.36, p = .555$ ).

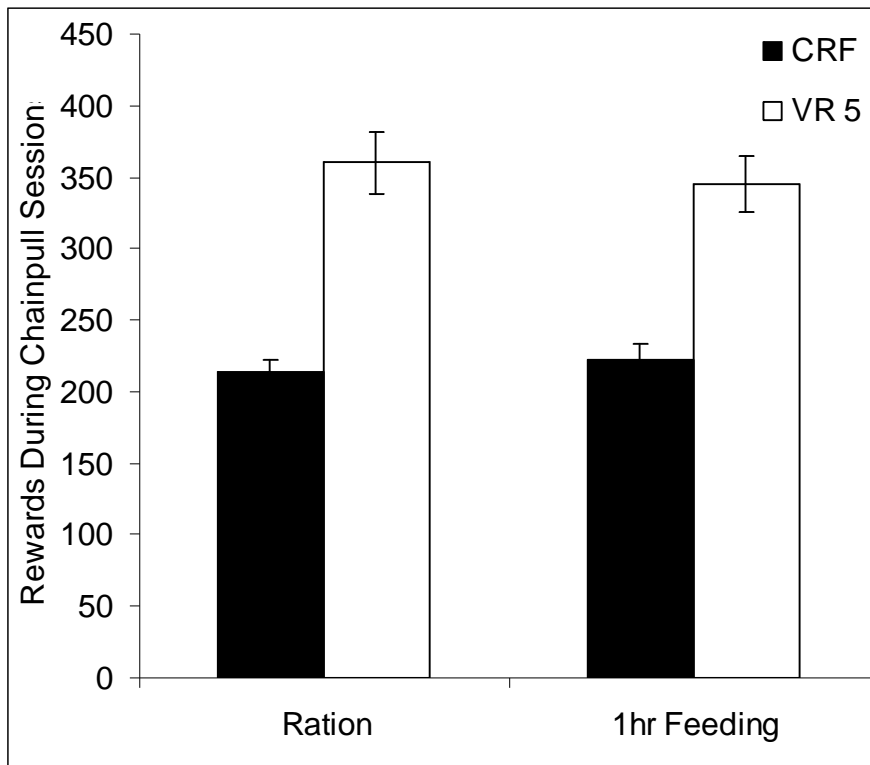


*Figure 6: Effects of feeding protocol on percentage of own free-feeding bodyweight.* The rats in the ration food restriction experiments had higher relative bodyweight when compared to the rats in the 1 hr feeding food restriction experiments. Percent bodyweight is calculated for each rat using the average bodyweight through the experiment while food restricted divided by the bodyweight measured before rats were food restricted at the beginning of the experiment.

#### *Chain Pull Phase*

The number of pellets required to reach discrimination criterion was higher for the VR 5 schedule compared to the CRF schedule, but did not differ based on food restriction (See

Figure 6). This interpretation is supported by the results of a 2 X 2 (food restriction X schedule) ANOVA using total number of pellets as the dependent variable. There was a main effect of schedule ( $F(1, 54) = 68.93, p < .001$ ) but no significant effect of food restriction ( $F(1, 54) = 0.04, p = .840$ ) or a food restriction X schedule interaction ( $F(1, 54) = 0.52, p = .474$ ).



*Figure 7: Pellets required during chain pull training.*

Rats were given chain pull training sessions on the RT (120-s) schedule until they reached the CS discrimination criterion of .90. Each session consisted of 20 pellets. The VR 5 reinforcement schedule required more pellets to reach the CS discrimination criterion compared to the CRF reinforcement schedule, while food restriction protocol had no significant effect.

## Chapter 5: Discussion

The non-reinforced groups demonstrated decreased lever pressing compared to the reinforced groups on the first lever press test day, as hypothesized but only in rats that were food restricted on the 1-hr feeding schedule. Although the second lever press test day with the CS was not administered in the ration food restriction experiments, a similar devaluation effect was found in both 1-hr feeding experiments, with the VR 5 schedule demonstrating lower lever pressing in the non-reinforced rats compared to the reinforced rats, while a similar pattern in the CRF schedule approached statistical significance. There were no differences on inactive lever pressing, suggesting that these differences in active lever pressing were not due to general activity.

The post-test light discrimination score was decreased in non-reinforced rats compared to reinforced rats in both VR 5 experiments, and a similar pattern approached significance in the 1-hr feeding CRF experiment. The post-test probe session was not given in the ration CRF experiment. There was no difference in discrimination scores when reinforced and non-reinforced groups were compared between experiments.

The VR 5 reinforcement schedule increased responding in non-reinforced rats during the extinction phase, compared to the CRF reinforcement schedule, as hypothesized, but only in the experiments with the ration food restriction. The VR 5 reinforcement schedule increased the number of pellets required to reach light discrimination criterion during the initial chain pull sessions, regardless of food restriction.

Baseline lever press rates were higher in the 1-hr feeding experiments compared to the ration experiments, and the CRF experiments had increased baseline lever press rates compared to the VR 5 experiments; there was no interaction between schedule and food restriction protocol. This contrasts with the average bodyweight data, that demonstrated an interaction between schedule and food restriction protocol showing that the rats in the VR 5 ration experiment had the highest average bodyweight, followed by the ration CRF experiment, that was higher than both 1-hr feeding experiments, that did not differ from each other. Reinforced rats weighed less than non-reinforced rats in the 1-hr feeding experiments, but not in the ration experiments. If baseline lever press rate was simply a function of bodyweight, then we would have expected no difference in baseline lever press rate between the CRF and VR 5 1-hr feeding experiments, or we would have expected a difference in baseline lever press rate between the reinforced and non-reinforced rats. Rats were assigned to the reinforced and non-reinforced conditions in a manner that equalized baseline lever press rate between the groups, so the observed test day devaluation effect was not likely to be attributed to differences in bodyweight.

The devaluation effect observed in the present study is similar to that observed in other studies of withholding reward (Salamone, 1986; Wise et al., 1978; Johnston et al., 2001; Shull & Grimes, 2006; Duarte et al., 2003; Olmstead et al., 2001), specifically in chain schedules (Johnston et al.; Olmstead et al; D'Andrea, 1969). This is also in accordance with other chain schedule devaluation studies that devalued the food reward by manipulating food restriction and using pre-exposure (Balleine, 1992), or re-exposure (Balleine et al., 1995; Dickinson et al, 1995) procedures.

The methods used in the current experiments devalued the CS by withholding reward during the isolated proximal link, similar to previous studies (Johnston et al., 2001; Olmstead et al., 2001). Devaluation of the CS has also been done by post CS LiCl injections (Igushi & Ishii, 2006) or by pre-exposure to the CS without reward (Killcross & Balleine, 1996). The current study differed from some other studies that devalued the primary reward itself by pre-feeding (Balleine, 1992; Dickinson et al., 1995; Dickinson et al., 2000; Shull, 2004) or post-reward injections of LiCl (Holland, 2004; Miles, Everitt, & Dickinson, 2003). Other devaluation methods seem to have depended on performing the operant response following injection of D2-like dopamine receptor antagonists (Salamone, 1986; Salamone et al., 2002; Wise et al., 1978; Pecina et al., 1997; Dickinson et al., 2000; Johnston et al.).

The current study was most like the work by Johnston et al. (2001) and Olmstead et al. (2001) in design, and the terms *taking* and *seeking* apply as they do in the current study. These studies trained rats on a two-link heterogeneous chain schedule, then withheld reward for some rats while administering the isolated proximal link, and giving response-contingent reward for taking responses in the other rats. The groups were tested in extinction with the seeking manipulandum in the chamber. As explained earlier, there are many important aspects to this design. First, the seeking manipulandum was different from the taking manipulandum that allows separate devaluation of the links. Second, the experiments used distinct actions (responses) on these distinct manipulanda to minimize potential action generalization effects. Third, inactive levers in the current experiment and Johnston et al. controlled for general locomotor effects, while Olmstead et al. used a

within-subjects concurrent design to control for this. Fourth, the rats were tested in extinction to prevent any CS reward re-evaluation effects, mentioned in the Introduction. For these reasons, it is unlikely that the decreased responding by the non-reinforced groups in the first lever press test day of the current study was due to generalization effects or re-evaluation of the food reward or CS as neither were presented to become re-evaluated. It was the *expected* value of the CS that was measured in the first test day.

The second test session with the CS was not used in the Johnston et al. (2001) or Olmstead et al. (2001) studies. D'Andrea (1969) evaluated the effect of giving the CS during extinction responding on a full heterogeneous chain schedule, and found increased responding on both links immediately following presentation of the CS using a temporal resolution of 30-s bins. Igushi and Ishii (2006) presented the CS during an extinction test and found enhancement of responding immediately after the CS presentation. Igushi and Ishii also demonstrated that it was possible, albeit difficult, to devalue the CS by using LiCl injections using a two-lever discrimination schedule. The current study used 5-min bins and did not have the temporal resolution to detect these rapid changes in responding on the second test day.

The second test day served as an addition to the first test day, with the added measurement of the effect of the CS on responding. If the CS was acting as a CR, then enhanced responding in the reinforced groups would be expected. If the CS was acting as a CR, then it would also be expected that the effect of the CR could decrease over the session. There was no evidence that the reinforced and non-reinforced groups were differentially affected over time during the second test session. Thus, if the CS was

acting as a CR it either was not subject to re-evaluation on the timescale tested, or the devaluation effect measured the first day was still measurable on the second test day. The current study was not able to determine which option was more likely.

The post-test light discrimination probe session was not used in the Johnston et al. (2001) or Olmstead et al. (2001) studies. This session measured the rats' ability to re-acquire light discrimination. If the CS was devalued as a discriminative stimulus, then we would expect non-reinforced rats to have had lower light discrimination scores due to the devaluation procedure, while the reinforced rats would have maintained their light discrimination scores. This session was subject to re-evaluation of the CS as the rats re-acquire light discrimination. Previous studies have failed to show an effect of reward devaluation in a reacquisition session (Dickinson et al., 1995; Balleine, 1992). Balleine used a procedure that at first looks like a first-order schedule of lever pressing for reward but it was later replicated by Balleine et al. (1995) who concluded that the magazine panel covering the food magazine was acting as a proximal link in a two-link chain schedule. In this manner, the reinstatement procedure was of the entire two-link chain schedule, not the proximal link as the current study performed. Dickinson et al. used a lever press RI 30-s schedule with no magazine panel. These studies did not separate links of a chain schedule for devaluation that may explain how they differ from the measured CS devaluation measured here.

Dickinson et al. (1995) have argued that the reacquisition phase removes devaluation effects by allowing concurrent re-evaluation that was too rapid to measure in the entire test session. However, the re-acquisition studies listed above (Dickinson et al.;



Balleine, 1992, 1995) did not isolate the links of a chain schedule. In this manner, the current study may be one of the first to demonstrate a reliable devaluation effect between links of a chain schedule (cf. Johnson et al., 2001). The current results differ from previous results in that motivational level, manipulated by food restriction in the current study, did not have an effect on responding in the re-acquisition test. It was found in the aforementioned studies that rate of responding in the re-acquisition test depended only on motivational state. This may have been due to different devaluation procedures, as the previous studies devalued the primary reward itself by exposing the rats to the food reward while in the satiated state, whereas the current study devalued the CS as a discriminative stimulus. This difference in results also may have been due to the isolation of chain schedule links in the current experiments. Finally, it may have been due to the measurements used. Dickinson et al. and Balleine (1992, 1995) used overall response rate, whereas the current study used a CS discrimination score that was specifically designed to be independent of overall response rate. If motivational state was increasing response rate overall, it would not be expected to alter the CS discrimination score. Indeed, discrimination score was unaffected by food restriction in the current study.

The present study found that the food restriction used through the entire experiment modulated the reward devaluation results; the groups that received the comparatively mild ration food restriction demonstrated no devaluation effect on the test day. Balleine (1992) demonstrated a measured devaluation effect in the distal link of a two-link chain schedule depends on testing in the same motivational state as training.

This finding was later replicated by Balleine et al. (1995), who extended the findings to demonstrate this pattern in a variety of chain schedules. Killcross and Balleine (1996) showed that measured latent inhibition effects were not due to general motivational arousal during testing, but were due to incentive learning during training regarding the reward value of the reinforcer based on the specific motivational state (food restricted vs. water restricted) during training.

Since the current study found performance effects during baseline lever press rates, the test day lever press rates cannot be directly compared. It is interesting to note, however, that since rats within each experiment were trained and tested in the same motivational state, it would be expected that reward devaluation effects would be found in all experiments, but this was not the case. An explanation of this finding awaits further study.

Balleine (1992) demonstrated that even satiated rats demonstrated the devaluation effect; however, Balleine devalued the primary reward itself, whereas the current study devalued the CS based on a criterion sensitive to performance effects (taking responses). It could be that in the milder ration food restriction condition, both the reinforced and non-reinforced groups were not greatly motivated to respond for reward, resulting in the non-reinforced groups reaching the extinction criterion through a combination of incentive learning and decreased performance (taking responses) during extinction due to lower motivational relevance of the CS. Indeed, the rats in the ration food restriction experiments demonstrated lower responding during baseline lever pressing that could be indicative of decreased motivational drive. Combined with the higher bodyweights in the

ration food restriction experiments, it could be that the motivational relevance was too low to properly devalue the CS using a performance-based criterion, such as taking responses. It could be that the non-reinforced rats in the ration feeding experiments did not have the drive to continue responding in the extinction phase, and thus did not receive a strong enough CS devaluation to be measured in the test day. It could be that the rats in the 1-hr feeding experiments had increased motivational relevance of the CS that maintained performance-based responding until the incentive learning devaluation had a strong enough effect to overcome the performance-based responding and the measured responding (taking responses) reached the extinction criterion. Learned and performance-based effects on responding were measured together as taking responses, and are virtually indistinguishable when analyzing one specific day in isolation. Learned effects would be demonstrated as a change in taking responses over days, whereas performance effects might be expected to be equal across days.

It should be noted that although the ration and 1-hr feeding experiments differed in bodyweight, bodyweight itself is not a pure measure of motivational state. There may be other measures of motivational state, such as plasma corticosterone or nutrient levels. Bodyweight also is affected by metabolic rate, not only food intake. Bodyweight was used in the current study because of simplicity and to prevent any undue stress which may be a result of other measures, such as the taking of blood samples.

The increased baseline lever press rate found in the CRF experiments, compared to the VR 5 experiments, was similar to the finding by Davison and McCarthy (1989) that increased reinforcement rate in the proximal link increased responding in the distal link

of a homogeneous chain schedule. The present study extended the finding to include heterogeneous chain schedules.

Limitations of the current study included the criteria used to separate the stages of the study. Because the rats in the VR 5 experiments required more pellets to reach light discrimination criterion, they arguably were trained more than the rats in the CRF experiments. The use of the light discrimination criterion was to ensure that all rats were performing at relatively the same rate by the end of each training phase. Otherwise, it could be that some rats moved on to the next phase when they were not performing at the same level as other rats. It is unlikely that rats that required more sessions to reach criterion of any phase were “more trained” than rats that required fewer sessions to reach the same criterion, as the responding prior to criterion was presumably not quite the same as responding near criterion. In short, it is argued that rats were not more *trained*, but rather they *required* more training to reach the same proficiency.

Another limitation was the puzzling finding that in the 1-hr feeding experiments; the reinforced group had a lower bodyweight than the non-reinforced group. It is not clear why this was the case, especially considering that all rats were given 60 min to eat, regardless of reinforcement condition. Baseline lever press rates were not different between reinforced and non-reinforced rats, in spite of the difference in the weights of the two groups, showing that weight did not predict lever press rate. One difference between the reinforced and non-reinforced rats that could explain the weight difference is that reinforced rats received pellets in the operant chamber, while non-reinforced rats did not. This is puzzling as the rats that were given *more* of an opportunity for food weighed *less*.

This weight difference could be due to a contrast effect between the palatable food reward in the operant chambers and the lab chow in the home cage, given 30-60 min after training. This contrast effect may have resulted in reinforced rats consuming less lab chow in their home cage to a degree that they consumed less total food overall.

Salamone et al. (2002) found that D1- and D2-like dopamine receptor antagonists decreased lever pressing for the same reward used in the present study, but increased consumption of lab chow concurrently available in the operant chambers. Although not directly comparable, the study by Salamone et al. demonstrates at least one case where food reward was not available and lab chow consumption increased, which could arguably explain the increase in weight of the non-reinforced rats in the 1-hr feeding experiments. The weights of the rats in the VR 5 ration experiment were higher than those of the rats in the other experiments. This may have been due to this particular experiment taking more days than the other experiments and rats gaining weight over days.

Because the second test session measured the devaluation effect in extinction of the primary reward, but in the presence of the CS, it is unclear if the devaluation effect measured on the second test day was due to persistence of the devaluation effect measured on the first day. If the CS were acting as a CR, we would expect it to selectively enhance or maintain responding in the reinforced group, but not in the extinction group. There is not substantial evidence from the current design to determine if the CS was acting as a CR in the second test day.

This research sheds light on the finding that subtle differences in food restriction can greatly change the results found in a devaluation paradigm. Whereas other studies have demonstrated the importance of motivational state by comparing satiated groups to food restricted groups (e.g. Balleine, 1992, 1995; Dickinson et al 1995) or groups that differed by 10-20% bodyweight (Pinkston et al., 2007), the current study demonstrated that even subtle differences in food restriction, such as difference of 5 percentage points between average bodyweight in the CRF experiments, can have strong effects on the obtained results. This should be considered for future research as one would legitimately conclude based on the ration experiments alone that the links in a heterogeneous chain schedule can be differentially devalued: that devaluation of the CS in the proximal link has no significant effect on responding in the distal link. It was only in the 1-hr feeding experiments that the devaluation effect was reliably found in the distal link.

Another implication of this research is that the two-link chain schedule used here is relatively robust to changes in the schedule of the proximal link. The devaluation effect was found in both 1-hr feeding experiments. One interesting difference was that the first test day showed significant devaluation in the CRF experiment and near-significant devaluation effect in the second test day with the CS, while the VR 5 experiment showed a significant devaluation effect on both days. It is possible that the CS can act as a CR in the second test day, which may be influenced by reinforcement schedule, but the data from the current study were inconclusive.

When the links of a heterogeneous chain schedule are isolated, it is possible to devalue the proximal link while not devaluing the distal link if the food restriction is

mild. This was evidenced by the CS devaluation measured in the ration VR 5 experiment post-test probe session while there was no measured devaluation effect in either ration food restriction experiment. This devaluation was measurable in a reacquisition session, at least when the measurement was not dependent on overall response rate, such as the post-test CS discrimination session and the CS discrimination score used in the current study.

It was demonstrated in the current study that increased reinforcement density in the proximal link of a heterogeneous chain schedule increases responding in the distal link. This pattern was found previously in a homogeneous chain schedule (Davison & McCarthy, 1989). The current study extended this finding to heterogeneous chain schedules.

Future studies should investigate the role of the CS during the second test session by measuring responding with sufficient temporal resolution to determine if responding increases briefly following presentation of the CS. This will allow the independent measurement of the direct effect the CS may be having on responding as well as allow comparison to any possible carryover devaluation effects measured in the first test day. It will determine if the devaluation effect measured on the second test day is similar to the devaluation effect measured on the first day, or if the CS was indeed acting as a CR.

The effects of dopaminergic drugs should also be investigated. The dopamine D2-like receptor antagonist pimozide has already been used in experiments very similar to the current study (Johnston et al., 2001), and other dopamine receptor antagonists have been shown to decrease operant responding in other schedules (Salamone, 1986;

Salamone et al., 2002). Specifically, the effects of drugs that are differentially selective for dopamine receptor subtypes should be investigated.

Unpublished research from our lab (Banasikowski & Beninger, 2007) using the conditioned activity paradigm has demonstrated a double-dissociation between dopamine D2 and D3 receptors. Both receptors are considered D2-like. It appears that D2 receptors are necessary for the learning of conditioned activity, whereas D3 receptors are necessary for the expression of conditioned activity. Administration of D2 receptor antagonists during the acquisition phase prevented the learning of conditioned activity. Administration of D3 receptor antagonists during the test session prevented the expression (performance) of learned conditioned activity. D3 receptor antagonists did not block the learning of conditioned activity when given during the acquisition sessions, and D2 receptor antagonists did not block the expression of conditioned activity when given on the test day. There are many methodological and theoretical differences between conditioned activity and chain schedules, but both can be understood as utilizing incentive learning. A D3 receptor partial agonist, that would also act as a partial antagonist, also blocked expression, but not acquisition, of conditioned place preference (Aujla & Beninger, 2005) and conditioned activity (Aujla, Sokoloff, & Beninger, 2002).

It would follow from the conditioned activity results that one would expect a D2 receptor blocker during the extinction phase would result in a learned devaluation, measurable on the first and second lever press test day and post-test probe session. This result was found in the Johnson et al. (2001) study. A D3 receptor antagonist would be expected to have little or no effect during the extinction phase but would be expected to



block the devaluation effect measured on the test days and post-test probe session. It may be that the D3 receptor antagonist may simply block the expression of chain pulling in the extinction phase, resulting in an apparent immediate devaluation which would not be measurable on the test day. The D2 receptor antagonist would be expected to have no effect on the first lever press test day, but could possibly result in decrease in responding in the later parts of the second test day, owing to a devaluation of the CS. The D2 receptor antagonist might have a learned devaluation effect occurring during the post-test CS discrimination probe session, but since the CS discrimination score is for the entire session, it lacks the temporal resolution to measure such an effect.

Amphetamine exposure enhances habit formation, making responses resistant to changes in reward (Nelson & Killcross, 2006). The effect of amphetamine during baseline lever press training could be investigated, it might be expected to enhance both lever pressing and chain pulling during baseline lever press sessions. This might result in resistance to extinction during the extinction phase, but may render the test day lever press resistant to the devaluation effect. Conversely, administering amphetamine during the normal 'extinction phase' could enhance lever pressing on the test day by an upward reward re-evaluation effect.

In conclusion, the reward devaluation effect in a heterogeneous chain schedule depends on sufficient motivational state, and is robust to changes in the schedule of the proximal link of the schedule. It is possible to separately devalue the proximal link without devaluing the distal link if the motivational state is not sufficient.

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