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### PAVLOVIAN CONDITIONED-REINFORCER TRAINING AS A MEANS OF

### REDUCING IMPULSIVE CHOICE

by

### Emma Visser

A thesis submitted in partial fulfillment of the requirements for the degree

of

### MASTER OF SCIENCE

in

Psychology

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Amy Odum, Ph.D Major Professor Gregory Madden, Ph.D Committee Member

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2024

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#### ABSTRACT

#### Pavlovian Conditioned-Reinforcer Training as a Means of Reducing Impulsive Choice

by

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Utah State University, 2024

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Impulsive choice is associated with a host of human maladaptive behaviors warranting investigations aimed at reducing impulsive choice. Pavlovian conditioning was explored in 22 male Wistar rats. The experimental group was exposed to a conditioned stimulus (CS) that signaled a large delay-reduction to an unconditioned stimulus (food), whereas the control group experienced a CS that was controlled by a random variable time (VT) schedule. Subsequently, a test of conditioned reinforcement was conducted, indicating a difference in conditioning between groups. Finally, a test of impulsive choice failed to find effects of Pavlovian conditioning on impulsive choice.

(37 pages)

### PUBLIC ABSTRACT

Pavlovian Conditioned-Reinforcer Training as a Means of Reducing Impulsive Choice Emma Visser

Impulsive choice is associated with a host of human maladaptive behaviors warranting investigations aimed at reducing impulsive choice. Pavlovian conditioning was explored in 22 male Wistar rats. The experimental group was exposed to a stimulus that signaled a large delay-reduction to an food, whereas the control group experienced a stimulus that was controlled by a random schedule that did not predict food. Subsequently, a test of conditioned reinforcement was conducted, indicating a difference in conditioning between groups. Finally, a test of impulsive choice failed to find effects of training on impulsive choice.

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Emma Visser

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#### INTRODUCTION

Impulsive choice is defined as preference for a smaller-sooner reward (SSR) over a larger-later reward (LLR; Ainslie, 1975). Impulsive choice is associated with human maladaptive behaviors including risky sexual behavior, obesity, tobacco use, alcoholism, gambling, and substance-use disorders (Amlung et al., 2017; Appelhans et al., 2011; Dixon et al., 2003; Johnson & Bruner, 2012). A causal relation between impulsive choice and these addictive disorders has not been established, but longitudinal data suggest that impulsive choice precedes substance use (e.g., Audrain-McGovern et al., 2009). This temporal sequence is consistent with the theory that impulsive choice increases susceptibility to addictive disorders; i.e., it is a trans-disease process (Koffarnus et al., 2013). The human and financial costs of addictions (e.g., substance abuse alone costs the United States \$600 billion and 67,367 lives per year; National Institute on Drug Abuse, 2018) provide a rationale for investigating experimental methods for reducing impulsive choice. For ethical and practical reasons, there are advantages to conducting this research in nonhuman laboratories.

The ideal experimental method of reducing impulsive choice, one more likely to be translated to human decision-making, would quickly produce large, reliable, and durable reductions in impulsive choice. In the animal laboratory, several approaches have been taken to reduce impulsive choice (for review, see Smith et al., 2019). Two of the most studied and contemporary approaches are *temporal training* (Peterson & Kirkpatrick, 2016; Smith et al., 2015) and *delay-exposure training* (Renda et al., 2018; Rung et al., 2018). Temporal training was born out of the finding that impulsive choice is higher in rats with imprecise interval timing (Marshall, Smith, & Kirkpatrick, 2014). During temporal training, rats learn temporal regularities of food availability under Fixed Interval (FI) or Variable Interval (VI) schedules of reinforcement. For example, rats learn that when the left lever is inserted, one food pellet may be acquired after completing an FI 10-s schedule (SSR). When, in a separate portion of the training phase, the right lever is inserted, an FI 30-s schedule controls the delivery of two pellets (LLR). Temporal training typically lasts 45 sessions and reductions in impulsive choice have been replicable (Bailey et al., 2018; Marshall et al., 2014; Panfil et al., 2020; Smith et al., 2019, 2015). From three representative studies in the temporal training literature, the median *Cohen's d* = 0.758; IQR = 0.513-0.943 (Bailey et al., 2018; Panfil et al., 2020; Smith et al., 2015). Temporal training with FI schedules produced a lasting reduction in impulsive choice when retested nine months after training, but lasting effects were not observed following VI training (Bailey et al., 2018).

*Delay-exposure (DE) training* provides rats with extended experience (typically 120 sessions) with a delayed reinforcement contingency. Specifically, a light-cued centerlever is pressed, the lever is retracted, and two pellets are delivered following a cue-lightsignaled 17.5 s delay. In three representative articles in this literature, DE training also produces large (median *Cohen's d* = 0.788; IQR = 0.630 – 1.153), replicable reductions in impulsive choice that remain significant when retested 4 months after training (Peck et al., 2019; Renda et al., 2018; Rung et al., 2018). Although the duration of DE training is far longer than temporal training, one experiment has reported that 60 sessions of DE training produced comparable reductions in impulsive choice (Cohen's *d* = 0.86), which remained significant when retested four months later (Renda et al., 2020). Given the number of sessions required to obtain lasting reductions in impulsive choice, exploring alternative training methods that may produce similar results with fewer sessions is warranted.

#### Pavlovian perspectives on temporal and delay-exposure training.

Stimuli that uniquely signal delay-reductions to an unconditioned stimulus (US), relative to the inter-US interval, will acquire conditioned stimulus (CS) function (Rescorla, 1988). Pavlovian learning is facilitated when the CS signals larger delay-reductions to the US (Gibbon et al., 1977; Gibbon & Balsam, 1981). The amount of delay-reduction is given by the *C:t* ratio, where *C* is the inter-US cycle time and *t* is the CS duration prior to US onset. As discussed by Shahan and Cunningham (2015), this ratio bears a formal resemblance to delay-reduction theory, a quantitative model of conditioned reinforcement (Fantino et al., 1993). A conditioned reinforcer is a previously neutral stimulus that has acquired the capacity to function as a reinforcer due to its contingent presentation with a US (Shahan, 2010). The speed of Pavlovian learning depends on the *C:t* ratio: larger values yield faster learning (Fantino et al., 1993; Gibbon et al., 1977; Gibbon & Balsam, 1981). For example, a *C:t* ratio of 60/18 = 3.33 is expected to require ~80 trials for the acquisition of a conditioned response, whereas a *C:t* ratio of 450/18 = 25 will cut acquisition time approximately in half.

Could Pavlovian learning play a role in the reductions in impulsive choice that are produced by temporal training and/or delay exposure training? Early *temporal training* experiments arranged free-operant procedures (no inter-trial blackouts), so leverinsertions and cue-lights did not signal delay-reductions to food; therefore, they could not acquire CS properties (Peterson & Kirkpatrick, 2016; Smith et al., 2015). However, in more recent temporal-training studies, a discrete-trial procedure has been employed, with fixed 60-s inter-trial blackouts that ended with a lever insertion and the initiation of either an FI 10-s schedule (SSR) or an FI 30-s schedule (LLR; Bailey et al., 2018; Panfil et al., 2020). Within this context, the insertion of the SSR lever signals that food is seven times more proximal than before, C:t = 7 (70:10). When the LLR lever is inserted, food is three times nearer, C:t = 3 (90:30). Given these ratios, the SSR lever likely acquires CS properties sooner than the LLR lever, and may evoke more sign-tracking (Domjan, 2003). If so, then inserting both levers during the test of impulsive choice may *increase* impulsive choice. Thus, Pavlovian processes would appear to play no beneficial role in the impulsivity-reducing effects of temporal training.

Does *delay-exposure* training employ procedures that may be profitably understood by considering Pavlovian learning? Within the delay-exposure training procedure, several stimulus events could function as a CS that evokes sign-tracking and/or function as a conditioned reinforcer. In a representative delay-exposure experiment (Renda et al., 2020), training began with an autoshaping procedure arranging an inter-reinforcer interval of 357.5 s and a CS duration of 32.5 s, resulting in a *C:t* ratio of 11. The CS in these autoshaping trials was a rear lever insertion, coupled with the corresponding cue light illumination. Renda et al. (2020) found that rats exposed to this autoshaping procedure alone showed a modest, but significant reduction in impulsive choice, relative to controls that received immediate food delivery upon a lever response during the CS. Thereafter, delay-exposure training continued with an operant contingency on the rear lever. The nominal inter-food interval (*C*) in these trials was 60 s. When the rear lever was inserted and corresponding cue-light was lit (the discriminative stimulus), the nominal time to food (*t*) was a bit over 19 s (an approximately 1.6 s response latency followed by a 17.5 s delay to food delivery), therefore C:t = 60:19.1 = 3.14. After the response, the lever was retracted and the cue light remained on, a stimulus configuration signaling only a slightly larger delay reduction to food than the light + lever stimulus (*C:t* ratio = 3.43). Because the *C:t* ratio during *delay-exposure training* ranged from 3.14 to 3.43, the rear-wall cue light and/or lever would be expected to acquire CS function after approximately 100 delay-exposure training trials (Gibbon & Balsam, 1981), or about two sessions. For rats assigned to the immediacy-exposure group, the lever + light stimulus signaled a much larger delay-reduction – from 60 s to approximately 0.6 s, *C:t* = 60:0.6 = 100. Thus, it is not surprising that the activation of the rear wall lever evoked faster lever-approach and lever-press behaviors in the immediacy-exposure rats (0.6 s) that the delay-exposure rats (1.6 s) during training.

But could these potential differences in Pavlovian learning influence behavior in the post-training test of impulsive choice? One reason for skepticism is that delay- and immediacy-exposure training was conducted on the rear wall of the chamber, whereas the testing of impulsive choice occurs on the opposing wall, with different levers and cue lights. The rear-wall lever and cue light are used only as a centering response that precedes the opportunity for a LLR or SSR choice. Thus, any Pavlovian learning that occurs during training is not expected to affect impulsive choice. The possible exception to this skepticism, however, occurs following a LLR choice. When that lever is pressed, the lever is retracted and the cue light remains lit – a stimulus arrangement that only delay-exposure rats have previously experienced, albeit on the rear lever. If this consequence of a LLR choice functioned not only as a CS, but also as a conditioned reinforcer (Baschnagel et al., 2007; Cronin, 1980; Palmatier et al., 2007) then this may play a role in greater preference for LLRs in delay-exposure trained rats (Mazur, 1998). That said, it is evident that delay-exposure training procedures have not been designed from a Pavlovian perspective. If they had been, the CS arranged during training would have more closely corresponded to the putative conditioned reinforcer arranged during the test of impulsive choice.

As previously noted, methods that quickly reduce impulsive choice have greater translational potential than long-duration training protocols. Temporal training requires ~45 sessions to produce lasting reductions in impulsive choice (Marshall et al., 2014) and delay-exposure training requires 60 sessions to produce large and lasting effects (Renda et al., 2020). By contrast, Pavlovian learning can be completed in just a few sessions when the *C:t* ratio is large (Balsam et al., 2010). The Renda et al. (2020) finding that a brief course of autoshaping was sufficient to significantly reduce impulsive choice suggests that further exploration of Pavlovian learning could benefit the goal of quickly reducing impulsive choice.

The proposed experiment took a systematic Pavlovian approach to training, with the goal of quickly establishing a CS that also has conditioned reinforcing properties. Specifically, Pavlovian CS training was arranged, with an 18-s compound light + noise stimulus that uniquely signaled a large delay-reduction to food – from an average (variable) inter-food interval of 450 s to a fixed 18 s delay (C:t = 25). Following training, rats were tested for a conditioned reinforcement effect. It was expected that rats exposed to Pavlovian training would respond to produce the CS significantly more than TRC trained rats, providing evidence for a conditioned reinforcement effect in Pavlovian trained rats only. Thereafter, in the test of impulsive choice, the CS/conditioned reinforcer was presented as the immediate consequence of choosing the LLR. I expected this consequence to enhance the value of the LLR (and hence, self-controlled choice), relative to a control group, for whom the compound stimulus has no CS/conditioned-reinforcing properties.

#### **METHODS**

#### **Participants**

A total of 28 male Wistar rats, approximately three months old at the start of the experiment, were used. Rats were obtained from Charles River Laboratory (Wilmington, MA) and singly housed in translucent polypropylene cages; dimensions were 46 (l) x 25 (w) x 21 (h) cm. Bedding (with beta chips or alpha dry/corn cob mix) and water were continuously available in the home cages. Rats were maintained at approximately 85% of their free feeding growth-curve weights (statistics obtained from Charles River). Rats were housed in a temperature and humidity-controlled vivarium with lighting on a 12/12 hour light and dark cycle, with lights turning on at 7:00 a.m. Half of the rats were randomly assigned to the Pavlovian training group and the rest to the truly random control (TRC) group at the start of the study. Group sizes were based on a power analysis and are similar to those used in previous studies that compared Pavlovian conditioning with a truly random control (Hyde, 1976; Kitaguchi, 2000; Rescorla, 1968).

#### Apparatus

Sessions were conducted in operant chambers controlled by a computer running Med-PC IV software (Med-Associates, St. Albans, VT). The interior of each chamber measured 30.48 cm wide x 20.96 cm tall x 23.13 cm deep and was situated in a sound attenuating cubicle. Each chamber was equipped with a house light, a white-noise speaker, head-entry detector (feeder area), ventilation fan, two cue lights 3.81 cm above two retractable levers (6.35 cm above the floor), with a food receptacle between them. Two nose-poke devices were installed on the left and right sides of the rear wall during some sessions.

#### Procedure

Rats first completed a single 32-min session in which they learned to eat pellets from the feeder area. Two pellets were delivered per US event according to a variabletime (VT) 2 min schedule with a constant-probability distribution of VT values (Fleshler & Hoffman, 1962).

### Lever training

Next, rats learned to press levers for food reinforcers. This was accomplished by baiting the rear-wall lever with an edible smear (i.e., peanut butter) prior to the start of the first session. At the beginning of each session, that lever was inserted into the chamber. Once a response on the rear-wall lever was made, the lever retracted and two food pellets were delivered. Upon head entry into the feeder to collect pellets, the lever was inserted back into the chamber. Sessions ended after 20 food deliveries or 1 hour. Training continued until rats earned 18 or more food rewards for two consecutive days.

#### Amount discrimination training

Subsequently, rats were exposed to amount discrimination training (one vs. three food pellets) which consisted of 60 trials per session (see Peck et al., 2020), divided into three 20-trial blocks with seven-minute blackouts between each block. The first six trials of each block were forced-choice trials (three on the left, three on the right; order

randomly determined). The subsequent 14 trials were choice trials. Trial onset was signaled by the insertion of the rear lever. A response on the rear wall lever resulted in either one (forced-choice trial) or two (choice trial) levers to be inserted. A response on a side lever caused immediate food pellet delivery (either 1 or 3 pellets depending on the lever). Failure to respond on a side lever within 20 s ended the trial and was recorded as an omission. Trials resulting in an omission were repeated until they were completed. Lever assignment for pellet amounts were counterbalanced within each group. Amount discrimination concluded when rats selected the large reward in  $\geq$ 90% of choice trials across two consecutive days.

#### **Pavlovian training group**

Procedures for this group were adapted from Holland, (2000). For all rats (Pavlovian and TRC groups) sessions began with the illumination of a diffuse cue light, external to the operant chamber, such that *C:t* ratios would be preserved during sessions and would not be confounded by how long rats were in the operant chambers. Within the chamber, rats began each session in the intertrial blackout, the duration of which was controlled by a VT schedule which ensured the average inter-food intervals was 450 s (rectangular distribution of values; see Holland, 2000). Blackouts were interrupted 18 s before each scheduled food delivery by the presentation of a compound CS (flashing houselight [0.25s on, 0.25s off] and constant presentation of white noise; L+N). After this 18-s L+N presentation, two 45-mg food pellets were delivered response-independently. Each session was composed of eight trials and terminated with the offset of the diffuse external cue light (Gallistel & Papachristos, 2020; Holland, 2000). Sixteen sessions of Pavlovian training were conducted. Elevation scores were calculated by subtracting the percentage of time spent with the head in the feeder during the 10 s preceding the CS from the percentage of feederentry time during the final 10 s of the CS. For example, if the rat spent 1 s in the feeder in the 10 s before CS onset (10% of the pre-CS interval) and 7 s in the feeder during the final 10 s of the CS (70% of the during-CS interval), then the elevation score = 70-10 = 60%. An elevation score of 0% would be indicative of chance responding (i.e., the rat spends the same amount of time in the feeder before the CS as it does during the final portion of the CS). Elevation scores are often used to measure acquisition, as higher elevation scores indicate the animal has learned the Pavlovian contingency (Holland, 2000). Sessions were conducted every other day (Gallistel & Papachristos, 2020).

#### Truly-random control (TRC) group

Rats assigned to the TRC group completed the same number of eight-trial sessions (16), on opposite days as the Pavlovian training group. TRC training was identical to Pavlovian training except that, during these sessions, rats were exposed to the 18-s L+N stimulus presented in a context of reinforcement, but such that there was no contingent relation between these cues and food. Thus, two independent VT 450-s schedules were used (rectangular distributions, as above). One VT schedule controlled the delivery of food pellets and the second controlled the onset of the L+N stimulus. As with the Pavlovian group, sessions onset was signaled with the illumination of a diffuse external cue light and offset was signaled with the extinguishment of the external cue light.

#### Assessment of conditioned reinforcing properties of the CS

To assess the conditioned reinforcing properties of the L+N stimulus, which was anticipated to differ across Pavlovian and TRC groups, all rats completed two conditioned-reinforcement test sessions following the training phase. For these sessions, two nose-pokes were installed on the rear wall; one on the left and the other on the right. Sessions started when the external cue light was illuminated, signaling session onset, and ended when this cue light was extinguished, signaling session termination. When the rat made a nose-poke response in the active nose-poke, the L+N stimulus was presented for 5 s. Responses in the inactive nose-poke had no programmed consequence. Active and inactive nose-pokes were randomly assigned. Food was never delivered during these sessions (i.e., extinction was in effect). Number of discrete nose-pokes, time spent in the nose-poke aperture, and number of CS presentations were recorded during the 40-min sessions.

#### **Return to training phase**

To counteract the effects of Pavlovian extinction for Pavlovian-trained rats in the prior phase, all rats returned to their assigned Pavlovian or TRC training conditions for three sessions.

#### **Impulsive choice test**

Finally, all rats completed a test of impulsive choice using a within-session increasing delay procedure (Evenden & Ryan, 1996). Sessions were composed of three 20-trial blocks with seven-minute blackouts separating each block. Trials began every 60 s, regardless of the choice made. The first six trials in each block were forced choice trials (three on the SSR lever and three on the LLR lever, presented in random order). Forced trials began with a rear lever insertion. A response on the rear lever retracted the lever and inserted either the SSR lever or the LLR lever. On forced SSR trials, pressing the lever retracted the lever and delivered one 45-mg pellet after a 0.01 s delay (the minimum resolution of Med-PC). On forced LLR trials, a lever press (a) retracted the lever, (b) presented the L+N stimulus, and (c) delivered 3 pellets after the delay as the L+N stimulus ended. Failure to respond within 20 s of lever presentation was recorded as an omission and the trial was repeated until all forced trials were completed.

The remaining 14 trials in each block were free-choice trials. Free-choice trials were identical to forced-choice trials except that both levers were presented after a rear wall lever press. A press on either lever resulted in retraction of both levers, and the sequence of consequences just described, depending on the lever chosen. Omitted free-choice trials were not repeated.

To ensure rats received exposure to the differing rewards amounts associated with each lever, every LLR selection (forced or choice) produced immediate LLR delivery during first block of each session. Delays to the LLR increased within sessions and across blocks according to the following sequence: 0, 15, and 30 s. Sessions terminated after 60 trials or two hours, whichever came first.

Choice was assessed at each delay (i.e., within session) for at least 10 sessions and until the following stability criteria were met: (a) there was no more than a 0.1 deviation in Area Under the Curve (AUC; see below) between the final 3 sessions and the 3 sessions prior (see Myerson et al., 2001), (b) over the last three sessions, there was no monotonic trend in percent LLR choice greater than 30%, and (c) over the last three sessions, percent LLR choice did not deviate by more than 30%.

#### DATA ANALYSIS

Due to side biases, six rats were removed from the study (four from the Pavlovian group and two from the TRC group), resulting in an analytic sample of 22 rats. The removed animals responded for the smaller reward (one pellet) in  $\geq$ 80% of opportunities across two days in the first block of the IC test when the LLR was not delayed.

In Pavlovian training sessions, the primary dependent measure was elevation score. A t-test was used to assess group differences in elevation scores, as Shapiro Wilk tests indicated elevation scores were normally distributed for both Pavlovian (p = 0.89) and TRC rats (p = 0.63).

Number of L+N presentations served as the main dependent measure for assessing the conditioned reinforcing properties of this contingent stimulus. A t-test was used to assess group differences in conditioned reinforcing properties because a Shapiro Wilk test indicated that, for both test days, the Pavlovian group (p = 0.21; p = 0.79) and TRC group (p = 0.47; p = 0.57) produced normally distributed data. Secondary measures for evaluating whether the L+N presentations may have acquired conditioned reinforcing properties included percent active nose-pokes by session and discrete active nose-pokes by session. Shapiro Wilk tests of normality revealed that for rats in the Pavlovian group, percent active nose-pokes were normally distributed on both session one and two (p =0.61, p = 0.24, respectively). The same was true for the TRC rats (p = 0.21, p = 0.68). Thus, t-tests were used to compare percent active nose-pokes by group. Pavlovian rats did not show normally distributed discrete active nose-poke responding in either of the two sessions (day one or two of the test for conditioned reinforcement) according to Shapiro Wilk tests (p = 0.03, p < 0.01). However, TRC discrete active nose-pokes were indicated to be normally distributed on both session one (p = 0.09) and two (p = 0.06) of the tests for a conditioned reinforcement effect. Therefore, t-tests were used to compare percent active nose-pokes between groups and Mann Whitney tests were used to analyze discrete active nose-pokes between groups.

In the tests of impulsive choice, percentage of LL choice at each delay, averaged across the stable sessions served as the primary dependent measure. Area under this choice curve (AUC) was used as a secondary measure. The area under the curve is calculated by summing the results of the following equation for each delay and indifference points:  $x_2 - x_1 [(y_1 + y_2)/2]$ , where  $x_1$  and  $x_2$  are successive delays and  $y_1$  and  $y_2$  are the indifference points associated with those delays (see Myerson et al., 2001 for more detail). Thus, larger AUC values indicate more self-controlled behavior (range = 0 to 1). Group differences in percentage of LL choice were evaluated using a 2 (group) x 3 (delays to the LLR) mixed-factors ANOVA. Because a Shapiro Wilk test indicated Pavlovian rats produced normally distributed data (p = 0.07) and TRC rats did not (p = 0.04), a Mann Whitney test was used to evaluate group differences in AUC.

#### RESULTS

Figure 1 displays the results of Pavlovian and TRC training. The figure depicts mean elevation scores ( $\pm$  SEM) across training sessions for both groups. For the Pavlovian group, elevation scores increased over 10 sessions, when they stabilized at approximately 50%. For the TRC group, elevation scores remained near 0% for the duration of training. The difference in mean elevation scores over the last five sessions was significant across groups; *t*(10) = 12.56, p < 0.001, indicating Pavlovian conditioning

was significantly different between the experimental group and the control group, but not that Pavlovian conditioning was reserved for the experimental group.

Figure 2 shows the outcomes of the test of conditioned reinforcement. During the first session, Pavlovian-trained rats activated CS presentations via active nose-poke responses significantly more than TRC trained rats; t(20) = 4.47, p < 0.001. On the second day of this assessment, both the Pavlovian trained group and the TRC group had reduced CS presentations, but Pavlovian trained rats still nose-poked to produce a significantly higher number of CS presentations, indicating the CS was a conditioned reinforcer for Pavlovian-trained rats, but not TRC rats; t(20) = 4.62, p < 0.001. The average proportion of active nosepokes in the Pavlovian group is significantly higher than the average proportion of active pokes in the TRC group on both day 1 and two of testing, t(16) = 2.15, p = 0.046; t(19) = 2.76, p = 0.012, respectively. Finally, active discrete nose pokes were significantly higher in the Pavlovian group compared to controls on both day one and two of the test for a conditioned reinforcement effect, U(10, 12) = 3.13, p = 0.002; U(10,12) = 3.40, p < 0.001.

These secondary measures support the conclusion that there was a significant difference in CS function as a CR for the Pavlovian rats compared to the control group.

The results of the impulsive choice test are displayed in Figure 3. The left panel shows mean percent LLR choice ( $\pm$  SEM) as a function of the delay to the LLR; data are from the final five (stable) sessions. The ANOVA revealed no significant main effect of group (F(1, 119) = 2.02, p = 0.158), a significant main effect of delay (F(2, 119) = 11.19, p < 0.0001, but no significant interaction between group and delay, F(2, 119) = 0.21, p = 0.812. No group differences were found at a 0 s delay to food reinforcers, t(101) = -1.18,

p = 0.243. When the LLR was delayed by 15 s, TRC trained rats had a significantly higher mean percent selection of the LLR than Pavlovian trained rats, t(85) = -4.25, p < 0.0001. Finally, when the LLR was delayed by 30 s, Pavlovian rats had a significantly lower mean percent LLR choice than did TRC rats, t(62) = -4.35, p < 0.0001. The second panel of Figure 3 depicts mean AUC by group averaged over the final 5 sessions of impulsive choice testing. A Mann Whitney test showed there was no significant difference in average AUC over the last five sessions between groups, U(10, 12) = -1.39, p = 0.166. Thus, no difference in impulsive choice between groups was observed. Data indicate that providing the CS as a consequence for a LLR choice did not result in significant impulsive choice changes between groups.

#### DISCUSSION

This study has three primary results. First, Pavlovian training produced significantly higher elevation scores compared to controls. Second, Pavlovian training was effective in conditioning a CS, as supported by the test of conditioned reinforcing properties. However, the CS was not effective in reducing impulsive choice.

Elevation scores were significantly higher in the Pavlovian training group than the TRC group. Because TRC elevation scores did not differ from zero, the L+N stimulus functioned as a CS in the Pavlovian training group only. Elevation scores were over 50% in the Pavlovian training group, consistent with Holland's (2000) data with comparable *C;t* ratios. Because procedures were based on Holland's and rats showed similar elevation scores, data suggest Pavlovian conditioning differed between groups.

Pavlovian rats activated the CS via active nose-poke significantly more times relative to the TRC group during the test of conditioned reinforcement. These results are

supported by similar findings by Bugelski (1938). Bugelski exposed rats to a levertraining procedure in which food delivery was preceded by a click. Subsequently, rats were assigned to two distinct extinction groups. In the control group, lever presses had no programmed consequence. In the training condition, a lever response produced a click. As in the present study, the training group responded to produce the CS significantly more than the control group. Arranging an extinction procedure, in which a response produces the CS, but not the UCS (i.e., food) is a widely used approach to determine whether the CS has acquired conditioned reinforcing properties (see Mazur, 2006). If the previously neutral CS stimulus has acquired conditioned reinforcing properties, higher rates of responding would provide evidence to suggest that the CS has acquired reinforcing properties itself. This is a critical outcome because it provides evidence that the CS has conditioned reinforcing properties for the Pavlovian group significantly more so than for the control group.

Finally, the Pavlovian-trained group did not make significantly fewer impulsive choices than the control (TRC) group, nor did their AUC differ significantly from the control group when the consequence of making a LLR choice produced the CS. Previous studies have had success finding differences in impulsive choice, following a different training procedure, using the same within-session increasing delay impulsive choice test procedure (Peck et al., 2020; Peck et al., 2022). Thus, this procedure is sensitive to training differences. Given TRC rats and Pavlovian rats did not show a significant difference in overall larger-later choice (AUC), we can conclude that Pavlovian training did not reduce impulsive choice.

One possible rationale for the lack of an effect of the CS on impulsive choice is that the test of conditioned reinforcement was an extinction procedure. Rats responded to produce the CS, but no US (i.e., food) was delivered. In order to mitigate extinction effects, rats were returned to their assigned groups and exposed to three additional days of training following the test of conditioned reinforcement. However, Bouton and Moody (2004) discuss retro-active interference, in which second-learned information interferes with first-learned information. It is possible that some learning occurred (i.e., diminished value of CS) from our conditioned reinforcement test due to a learning history that the CS does not always predict the US. Therefore, lack of effect of the intervention could be a result of employed testing procedures present in the present experiment.

Another consideration for the failure to observe an effect of the CS on impulsive choice is the mis-match between the expected size of food delivery and the actual food delivery, The Rescorla Wagner model of learning posits that (a) learning occurs when what happens on a trial does not match was is expected and (b) expectation on any given trial is based on the predictive value of the stimuli present (Rescorla, 2008). During training, Pavlovian rats learned that the CS was worth a US delivery of two pellets. Next, during the test of conditioned reinforcement, rats learned the CS is worth no pellets. Finally, in the test of impulsive choice, a LLR choice (worth three pellets) produced the CS for the duration of the delay. If rats learned the CS was worth two pellets, and the delay was more than twice as aversive as one pellet, according to the Rescorla Wagner model, the CS could have predicted a less valuable choice, at least during initial IC sessions.

This study has at least three potential limitations. First, without an impulsive choice pretest, it is impossible to know how much impulsive choice changed for either the Pavlovian training group or the TRC group (Fox, Visser, & Nicholson, 2018). That is, any post-training group differences could be due to increased impulsive choice in the Pavlovian training group or reduced impulsive choice in TRC group. Data from the impulsive choice test supports this hypothesis, as the TRC group was more self-controlled at each delay, including the 0 s delay.

It could be that the intervention had no effect on intertemporal choice. That is, it is possible that training a CS to predict a reward does not reduce the aversiveness of delay. During training, Peck et al. (2019) exposed the experimental group to a CS (light) following a response indicating a delayed reward. In the present study, the onset of the CS predictive of a delayed reward required no response. Although the test of IC was identical in both Peck et al. (2019) and this study, rats in this study did not experience any consequences of their responses during the training phase. It could be that without a response/consequence relationship during training, intertemporal choice is not affected by the CS.

A final limitation worth discussing is the inclusion of male rats only. Weafer and deWit (2014) found no sex differences in impulsive choice for food rewards. However, it is possible there are sex differences in how conditioned reinforcement impacts impulsive choice across sexes.

Future laboratory studies should focus on several areas. A pretest of impulsive choice, before exposure to Pavlovian training, would rule out the possibility that any post-training group differences were due to an increase in impulsive choice for TRC

group. Additionally, in order to determine whether Pavlovian training differentially impacts impulsive choice in males and females, testing the Pavlovian training procedure to improve self-control could be valuable. While the results of this study are not consistent with the hypothesized outcome, further investigation of Pavlovian arrangements aimed at reducing impulsive choice could prove valuable in terms of making efficient and lasting reductions in impulsive choice.

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### FIGURES

### Figure 1

Illustration of Training Results



*Note.* Mean elevation scores by session for the Pavlovian training group and TRC group. Error bars display SEM.

### Figure 2

Mean CS Presentations by Training Group



*Note.* Figure 2 displays mean CS presentations by group during the test of conditioned reinforcement on sessions 1 and 2, respectively (Pavlovian compared to TRC). Error bars reflect SEM. Individual data points reflect individual animal performance.

### Figure 3

Impulsive Choice Test Results



*Note.* Panel 1 shows mean percent LLR choice by delay to food during the test of impulsive choice for the Pavlovian training group and TRC group over the final 5 sessions. Panel 2 displays mean AUC for both groups for the last 5 sessions. For both panels, error bars display SEM. In panel 2, individual data points reflect individual animal performance.