EFFECTS OF ACUTE CANNABINOID AGONIST WIN 55,212-2 ADMINISTRATION ON REPEATED DOWNSHIFTS IN AUTOSHAPING WITH RATS

by

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ABSTRACT

The importance of cannabinoid receptors has risen in recent years due to the increasing number of states that have legalized marijuana. Previous research from our lab has explored coping with multiple instances of reward loss when exposed to large, chronic doses of the cannabinoid agonist WIN 55, 212-2 (WIN, 10 mg/kg). When chronically exposed rats received a consummatory successive negative contrast (cSNC) downshift from 32% to 4% sucrose, they were less able to cope with the subsequent autoshaping downshift of 12 pellets to 2 pellets. Additional autoshaping research from our lab has shown multiple downshifts in autoshaping to be successful in obtaining contrast effects. The present research combined this procedure with occasional acute doses of WIN (1 mg/kg) to determine if only one kind of downshift experience, autoshaping, was sufficient to produce less coping efficacy if repeated. Rats were randomly assigned to either WIN or vehicle control groups, and then trained in acquisition with discrete lever presentations where one lever was always followed by the delivery of 12 pellets, and a second lever was always followed by 2 pellets. After acquisition, rats received downshift sessions once per week, wherein the lever previously associated with 12 pellets was downshifted to 2 pellets. Prior to each of 4 downshift sessions, rats received intraperitoneal injections of either WIN or vehicle solution. Lever presses to each lever during discrete "forced choice" and simultaneous "free choice" trials, and head entries into the cup where food was delivered, or "goal entries," were both recorded to assess preference and explore downshift effects. Although acute WIN administration did not affect lever preference relative to vehicle controls, it did result in decreased lever pressing in favor of goal tracking during the downshift. Therefore, WIN seems to encourage rats to be more focused on the outcome instead of responding to signals for the

outcome, which may have implications for reducing impulsive behavior despite extensive training.

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Incentive Relativity

Tinklepaugh (1928) was one of the first researchers to show a nonhuman example of animals forming an expectation for a reward and reacting subsequently. He designed an experiment in which instead of giving monkeys their expected food reward of a banana, which they view as a higher food item, he gave them lettuce which they view as a lower food item. The monkeys reacted negatively and either outright rejected the lettuce or even threw it at the researchers. The monkeys had formed an expectation of what food reward they would receive (banana) and subsequently reacted when this expectation was violated due to a reward downshift (lettuce).

Crespi (1942) also looked into expectations and reward loss, but instead of doing it in a qualitative way like Tinklepaugh did, he looked at it in a more quantitative way. He would have rats run down a runway and at the end of the runway there would either be a large amount of food pellets or a small amount of food pellets. Once the rats got used to this, he would give them the opposite amount. The rats that had the downshift from the large amount of food pellets to the small amount had their average latency decreased in comparison to the rats that consistently received just the small quantity of food pellets. It took the rats longer to run down the runway and get the food. This behavioral shift was attributed to behavioral disruption caused by negative emotion (frustration). This effect is known as a negative contrast effect and one way to study this is by using an autoshaping procedure.

Autoshaping in rats

Autoshaping is a Pavlovian situation in which a retractable lever is presented as a conditioned stimulus (CS) for a fixed period of time, and then, upon its retraction, a food reward, unconditioned stimulus (US), is immediately dispensed into a food cup. Rats are not required to

press the lever, but after a few pairings of the lever and food reward they begin to approach and interact with it. Responses to the lever are recorded as a measure of the CS's strength as a signal for food reward. This behavior is known as sign tracking. Goal tracking is also measured; this behavior involves inserting the head into the area where the food is delivered. Sign and goal tracking act as measurable markers for a US expectation. For example, increased lever pressing occurs when the expected food reward is signaled, but not presented (Dudley & Papini, 1995, 1997).

Autoshaping allows for the experimenter to control each stimulus presentation so that conditions are made equal among all of the animals. This is especially important for when there is more than one stimulus assigned to a specific reward level. Two levers can be used, designating one to be followed by a large and the other followed by a small reward. After training with these two levers in single-lever trials, simultaneous presentations of both levers allow for a free-choice trial in which the animals can opt for one of the two. This way, it can be seen if the animal has a preference for one lever over the other. Testing with a free-choice trial necessitates that learning opportunities are equal in training, including the number of total lever presentations for all of the animals in order to compare preference among conditions.

Conrad and Papini (under review) used this procedure with two levers, one signaling 12 pellets and the other lever signaling 2 pellets. The authors ran the rats in 10-minute sessions and within these sessions the rats would receive 3 trials of each lever in a random order. After 18 sessions, they gave the rats one probe trial at the very end of the session. Both levers were presented at the same time and a food reward was not given afterwards. This trial looked to see if the rats had a preference for one lever over the other based on their reward expectation. After 6 sessions with this probe trial, the rats were responding to the 12 food-pellet lever significantly

more than to the 2 food-pellet lever. The 12 food-pellet lever was then downshifted to 2 food pellets on Session 19. Rats exhibited a preference for the unshifted, 2-pellet lever over the downshifted lever, even though both levers were now paired to the delivery of 2 pellets. The probe trial allows for the effects of frustration due to reward loss to be objectively observed.

Cannabinoids

Cannabinoids are neuromodulators related to the active component of cannabis or marijuana. Research has shown that cannabinoids can attenuate physical pain, that is, act as analgesics. It has also been found that cannabinoids can attenuate psychological pain or frustration as well. Previous research done in our lab looked at how the brain coped with reward loss when exposed to large, chronic doses of the cannabinoid receptor 1 and 2 (CB1, CB2), agonist WIN. An agonist is a chemical that binds to a receptor and activates it to produce a biological response. The hypothesis was that successive, chronic doses of WIN would downregulate the cannabinoid receptors. Two different types of downshifts or reward loss were used: a consummatory negative contrast (cSNC) downshift with sucrose and an autoshaping downshift with food pellets, as described above. It was found that when the chronically exposed rats received a cSNC downshift from 32% to 4% sucrose, they were less able to cope with the subsequent autoshaping downshift of 12 food pellets to 2 food pellets. This may have implications for humans that are exposed to chronic marijuana use in that they may be at risk in situations involving severe or repeated reward loss.

Current Research

Previous studies have shown that Pavlovian autoshaping training may involve incentive relativity effects (Papini et al., 2001) and an effective way of bringing out these effects is by free-choice trials (Conrad & Papini, under review). These findings, in combination with recent

research done with cannabinoids and coping led to the current study. The hypothesis for this thesis is that the CB1/CB2 agonist WIN will attenuate the effects of frustration after multiple reward downshifts in autoshaping. Previous research done by the lab used two different types of downshifts in the procedure, cSNC and autoshaping. The question is whether the decrease in coping efficacy is due to the two different types of downshifts or because there were multiple downshifts.

Method

Subjects

Subjects were 16 male Wistar rats bred at the TCU vivarium. Rats were housed in individual, wire-bottom cages. The cages included an enrichment object and allowed for constant water access. The colony room was at a controlled temperature (22-23 °C) and humidity level (50-65%), while being maintained on a 12 h light/dark schedule (light on from 07:00 h to 19:00 h). Rats were gradually food deprived to 81-84% of their ad libitum weight once they were 90 days old. To preserve the 81-84% food deprivation level, rats received supplementary food 30 min after each daily section.

Apparatus

Four individual sound-attenuating cabinets that contained standard operant chambers (MED Associates, St. Albans, VT) were used for conditioning. The measurements of the conditioning boxes were 20.1 x 28 x 20.5 cm (W x L x H) and the floor was made up of 0.4 cm diameter stainless steel bars that were 1.6 cm away from each other. To collect any droppings, a pan filled with corncob bedding was placed underneath the bars. 45-mg reinforcement pellets (Bio-Serv, Flemington, NJ) were dispensed from an external pellet hopper into a food cup. Photocells were equipped into the food cup to capture head entries (goal tracking) automatically.

Located 1 cm at either side of the food cup and 6 cm from the floor were two stainless steel retractable levers. Within the sound-attenuating enclosures, a fan to promoted airflow, a house light (GE 1820) provided diffuse light, and a speaker delivered masking white noise. The fan and speaker registered a combined contextual noise of 80.1 dB (SPL, scale C).

Procedure

Once rats reached their food-deprived weight, they began their first day of autoshaping. The presentation of a lever acts as a signal for food, but since this is a Pavlovian task interaction with the lever is not necessary. Nevertheless, the rats will interact with the lever as if it were a piece of food by biting, licking, and manipulating it. In anticipation of the food reward, the rats will begin poking their heads into the area where the food is going to be dispensed (goal-tracking behavior). The first phase of the experiment, acquisition training, lasted 7 sessions, 1 session per day, followed by the first preshift phase (Preshift 1). Preshift 1 lasted 6 sessions and mirrored acquisition training. Each session has 6 trials where one of two levers were presented for 10 s. A total of 3 right levers and 3 left levers were presented for each session; levers were counterbalanced and randomized. Retraction of a lever coincided with the presentation of food. One lever was designated as a high food reward (12 pellets) and the other lever was designated as low food reward (2 pellets). Half of the rats had the right lever as the high reward and the other half had the left lever as the high reward as part of the counterbalance. At the end of each trial (i.e., when the lever was retracted), 12 or 2 45-mg pellets were automatically dispensed depending on the lever. After the 6 sessions of Preshift 1, rats had their first session of postshift (Postshift 1).

Postshift 1 was identical to acquisition and Preshift 1 in how the levers were presented, but this time the high reward lever was downshifted to a low reward (12→2 pellets) and the other

lever stayed constant. 30 min before autoshaping, half of rats received an intraperitoneal injection of WIN, 1 mg/kg dose, and the other half received the vehicle (70% DMSO, 30% sterile saline). At the end of the 6 forced-choice trials, rats were presented with a free-choice trial where both levers were presented at the same time for 10 s and sign-tracking responses were recorded independently. This 7-session preshift/postshift cycle was repeated 3 times to gather data for a total of 4 downshifts.

Table 1

Behavioral Training Procedure

Repeated 3 Times

n= 16	**Acquisition** (7 sessions)	**Preshift 1** (6 sessions)	**Postshift 1** (1 session)
8	R12, L2	R12, L2	Downshift **R2**, L2 Choice: R vs. L
8	L12, R2	L12, R2	Downshift **L2**, R2 Choice: R vs. L

Note. R: right lever. L: left lever. 12 and 2: number of pellets per trial. Reward size counterbalanced across R and L lever. The shifted lever (downshifted) appears in bold.

Results

Postshift free choice trial data was separated into the average of Postshift 1 and Postshift 2 and the average of Postshift 3 and Postshift 4. Postshift free choice trial data refers to the trial presented at the end of the postshift sessions when both levers are presented simultaneously to the animals. The rats had a choice between responding to the downshifted 12-2 pellet lever or the unshifted 2-2 pellet lever. Figure 1 shows a graphical representation of the Postshift free-choice data. Overall, the animals had a preference for the unshifted 2-2 pellet lever. There was a tendency for WIN to eliminate the preference for the unshifted 2 pellet lever in the later

postshifts (z = -.561, p = .575) in comparison to the earlier postshifts (z = -1.472, p = .141); the later postshift cannabinoid animals are less responsive to the downshift.

Response bias was looked at to determine if the animals had a bias for sign tracking (lever-pressing) or goal tracking. Response bias was calculated by subtracting goal tracking from lever pressing. The average of Postshift 1 and Postshift 2 and the average of Postshift 3 and Postshift 4 in the postshift free-choice trials are presented in Figure 2. For the most part, the animals preferred lever pressing over goal tracking. Cannabinoid animals in the earlier postshift did not have a preference for lever-pressing or goal tracking. There was a significant difference between cannabinoid animals and control animals in response bias in both the earlier postshifts (z = -2.012, p < .05) and the later postshifts (z = -1.956, p < .05) . Cannabinoid animals are doing less lever pressing in comparison to the control animals. WIN seemed to have shifted the balance from sign tracking to goal tracking.

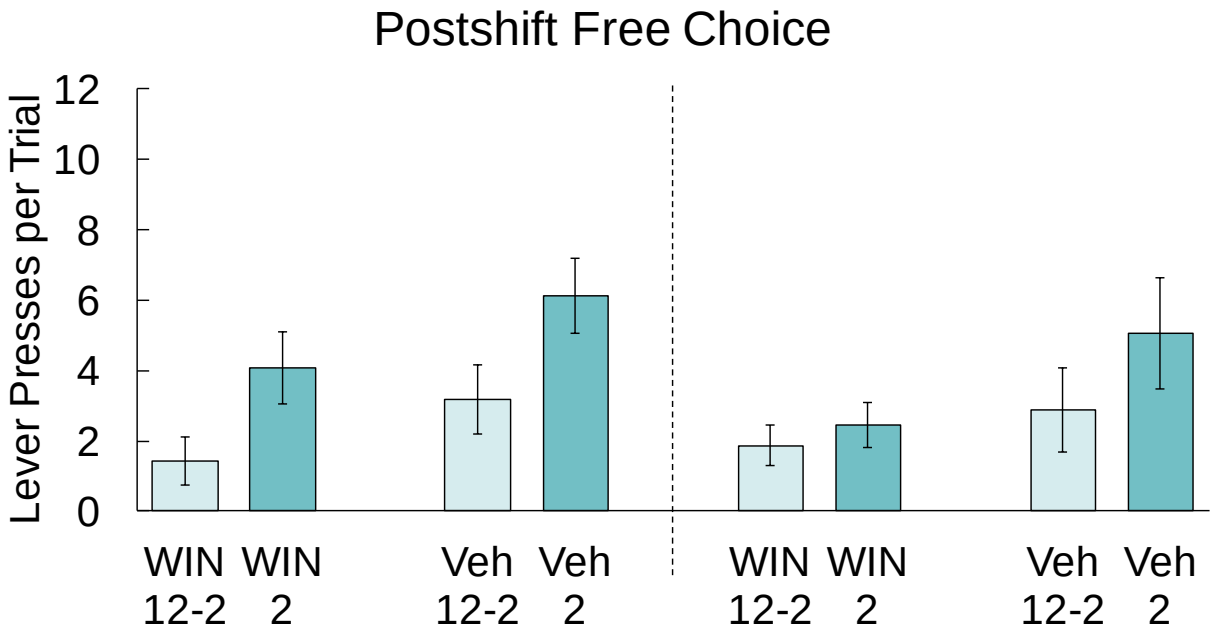

Figure 1. Responses per trial to each lever across all 4 Postshift free choice trials.

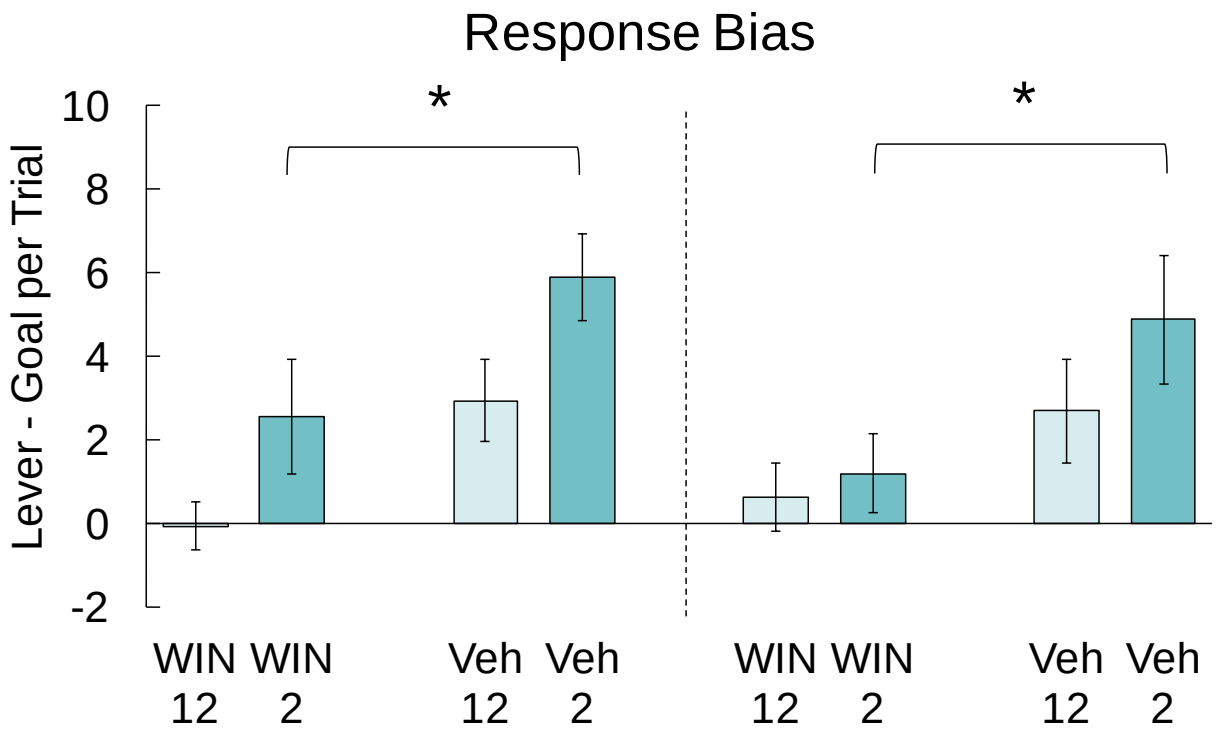

Figure 2. Goal tracking responses subtracted from lever pressing responses per trial across all 4 postshift sessions. (Note: * denotes $p < .05$)

Discussion

These results suggest that there is a difference between the animals that received the cannabinoid agonist and the ones that received the vehicle. More noticeably, there was a difference between the cannabinoid animals in the later postshifts in comparison to the earlier postshifts. The cannabinoid animals began to eliminate their preference for the unshifted lever in the later downshift in comparison to the earlier postshift and control. This suggests that the later postshift animals are less responsive to the downshift; they are not responding as they were in the earlier postshift.

The size of the sample is small at only 16 animals. The experiment would need to be reproduced with additional animals to affirm the conclusions drawn from the data. Postshift free choice trial data that demonstrated that later cannabinoid animals have eliminated preference for

the unshifted lever was not found to be significant in the current experiment. Possibly, this effect would be found significant with the addition of more animals to the experiment.

WIN was also found to have shifted the balance from lever pressing to goal tracking meaning that the animals were more interested in the food than the signal for food. Since increased sign-tracking has been linked with addictive behavior (Morrison, Bamkole, & Nicola, 2015), the trend towards increased goal-tracking for WIN animals in comparison to vehicle controls suggest CB receptor activation may make animals behave less impulsively.

Many states are legalizing medical marijuana, so it is important to continue research into the consequences of marijuana usage

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