The Effect of Chronic Corticosterone Administration During Abstinence on Heroin Seeking in Male Rats *

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Drug addiction remains a prevalent issue. Of the cycle of drug addiction, the most problematic phase seems to be the high rates of relapse after abstinence or treatment. Stress is one trigger of relapse. Recent findings suggest chronic food restriction, a mild stressor known to increase corticosterone, induces an increase in heroin seeking. Chronic administration of corticosterone during the abstinence period was used to analyze heroin seeking behavior. Male rats were trained to self-administer heroin for 10 days then separated into three groups (water, vehicle, corticosterone) for the duration of the 15-day abstinence period. Following abstinence, a heroin-seeking test was conducted under extinction conditions. We found no evidence to support the hypothesis that corticosterone affects heroin seeking, suggesting that the augmentation in heroin seeking induced by chronic food restriction is not mediated by corticosterone.

Drug addiction has multiple harmful effects. Substance disorder not only deteriorates the users' physical and mental well-being, but also impacts their families, friends, and the community [1]. In addition, there are large-scale societal costs, such as over-packed health institutes and public hazards. Drug addiction is a chronic disorder that is often described as a vicious cycle characterized by compulsive drug seeking, interwoven with recurrent periods of drug abstinence, and relapse [2,3]. Substance addiction produces long-lasting neurophysiological changes that persist even when there is prolonged abstinence. For instance, disrupted corticotrophin-releasing factor (CRF) release in the extended amygdala, an area key in drug reinforcement [4]. Changes induced in key regions, accompanied by environmental and psychosocial factors, then contribute to relapse [2]. Heroin is a highly addictive drug. The death rate among heroin users is 50 to 100 times greater than the rate of the general population, according to Smyth [5]. This illicit substance carries with it a very high rate of relapse, reaching up to 91% [5]. Zickler and colleagues reported that one-quarter of the people recovering from addiction have relapsed after 15 years of abstinence [6]. To date, the neurobiological mechanisms that mediate relapse to addictive drugs remain elusive, and this research project aims to bridge this gap.

Three key factors trigger relapse to drug use. The first is exposure to the drug itself after a period of abstinence. For example, abstinent drug users' risk of relapse significantly increases if they come in contact with the illicit drug [7–9]. Secondly, cues previously associated with drug use are potent triggers for reuse [6]. Cues like the original drug-taking context, the individuals that surrounded the drug user during consumption, as well as the materials or tools used to once administer a drug, are strong associations that can lead to relapse. Lastly, stress is commonly associated with drug relapse. Recovering drug users are vulnerable to a plethora of acute or chronic stressors, such as high periods of stress at work or traumatic life events [7–10]. Studies

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used to understand the effect of stress on drug relapse commonly use food restriction as either an acute or chronic stressor. In humans, chronic food restriction is known to increase drug taking and craving [11] whereas acute food deprivation has not been shown to affect the number of cigarettes smoked [12]. Food restriction provides an interesting avenue for studying stress-induced relapse. A potential mechanism explaining the association between stress and relapse is the activation of the hypothalamic-pituitary-adrenal (HPA) axis [9,13]. The HPA axis begins with the stimulation of the hypothalamus which then results in a cascade of events leading to the release of the non-human primate glucocorticoid [22].

A potential trigger of the HPA axis is dietary manipulations. Interestingly, the effects of dietary manipulations on drug-taking and seeking in rats are similar to those observed in human addicts (i.e. readily self-administering a drug). Research suggests that an increase in drug-taking is related to a decrease in body weight, and a lower bodyweight led to more drug intake [19]. More specifically experiments conducted by D'Cunha and colleagues demonstrated that chronic food restriction increased heroin seeking by 250% compared to rats that were sated [10]. Taken together, these results support the idea that dietary manipulation increases drug-taking and drug seeking in rodents.

Previous research has established the relation between chronic food restriction and increased drug intake in both human and rodent studies. However, the underlying neural mechanisms involving food restriction-induced augmentation of heroin seeking behaviour remain elusive. As previously mentioned, research has indicated that chronic food restriction during abstinence increases subsequent heroin seeking [10]. Of particular interest, is research by Carr and colleagues that found that animals that were food-restricted for 14 days compared to a food-sated control had elevated corticosterone concentration levels [20]. This experiment aims to further explore the role of chronic corticosterone by mimicking the elevated corticosterone effect observed following chronic food restriction. This goal will be achieved by chronically administering exogenous corticosterone during abstinence following heroin self-administration training. We hypothesize that this chronic administration of corticosterone will increase heroin seeking following abstinence.

Method

Animals

Twenty-three male Long Evans rats served as subjects for this experiment (Charles River, Raleigh, New Jersey, U.S.A.). Upon arrival, all rats weighed between 250-275g. Rats were paired housed in plastic shoebox cages during the acclimatization period for a week in the Animal Care Facility (ACF) on a reverse light/dark cycle (9:30 am lights off – 9:30 pm lights on). For the entire duration of the experiment, all rats had *ad libitum* access to food (Agribran Purina Canada Inc., Woodstock, Ontario) and water. Following acclimatization, rats underwent surgery followed by a two-day recovery, at which time they were housed in cages separately. After two days of post-operational recovery, they were transferred to the operant self-administration chambers for training.

Intravenous catheterization

Rats were implanted with an intravenous (i.v.) Silastic catheter (Dow Corning, Midland, MI, USA) 3 cm into the right jugular vein, which was held in place with silk sutures, as previously described in [10].

Apparatus

Training involved 10 operant conditioning chambers (Med Associates Inc., St. Albans, Vermont, USA; 32.0 cm X 24.0 cm x 25.0 cm) enclosed in sound-attenuating boxes equipped with a fan. Each chamber contained a red house light , a food hopper, and a water bottle. The chamber included two levers. The active lever is located 5 cm above the floor and is installed on the wall opposite to the houselight. The inactive lever is located 5 cm above the floor on the same wall as the active lever. An infusion pump (Razel Scientific Instruments, Stamford, CT) was installed inside the sound-attenuating cabinet. A cue light and a tone generator (2.9 kHz; 10 dB) are located directly above the active lever. The active lever was paired with a drug infusion. Pressing on the inactive lever did not result in a drug infusion, although responses were still recorded. This record served as a control for baseline, non-reinforced operant responding. Tygon tubing (Saint-Gobain, Courbevoie, France) was attached to a swivel (Lomir Biomedical Inc., Notre-Dame-de-l'Île-Perrot, QC, Canada) that was connected to a 20.0 ml syringe mounted on the infusion pump, through which the drug was delivered.

Drugs

Heroin (diacetylmorphine HCl; provided by the National Institute for Drug Abuse, Research Triangle Park, NC, USA) was prepared by dissolving it in 0.9% sterile saline (5.0 mg/ml). Based on the body weight of each rat, this solution was further diluted with 0.9% saline in order to yield 0.1 mg/kg/infusion.

Corticosterone (Sigma, Oakville, ON., Ca) was dissolved in 99.99% ethanol with the use of a sonicator. This solution was further diluted with water, yielding a 2% concentration of Ethanol and a concentration of 300 μg/ml corticosterone.

Procedure

Self-administration training. First, rats were placed in the operant chamber for a 24 h habituation period. Following the 24 h habituation period, rats underwent 10 days of heroin self-administration training. Rats were exposed to three 3 hr training sessions per day. An interval period of 3 hr separated each training session. Each training session started with the illumination of the house light, followed by the extension of the active lever and the activation of the cue light and the cue tone. This sequence lasted 30 s, or until a response on the active lever was made. Training involved a fixed-interval 20 (FI - 20) schedule of reinforcement with a 20-s timeout period. That is, pressing on the active lever resulted in a 0.1 mg/kg infusion of heroin over 12 s, as well as the activation of the cue light and tone and turning off of the house light. Furthermore, pressing on the active lever resulted in a 20 s timeout period. This timeout ensured that the rat would not overdose. Any lever presses made during the 20 s interval following the initial response did not result in an additional infusion, but the responses were recorded. The inactive lever is adjacent to the active lever and there are no programmed consequences. All responses on the inactive lever were also recorded.

Drug Withdrawal. Upon completion of 10 days of self-administration training, rats were removed from the operant conditioning chamber and moved to the ACF. Rats were individually housed for 24 h and underwent a drug washout period. During this washout period, rats had *ad libitum* access to food and water. Next, rats were separated into three treatment groups [water, vehicle (2% ethanol in water), corticosterone] and went through forced abstinence over 15 days.

Heroin Seeking Test. On the test day (15th day of the forced abstinence period), rats were brought back to the operant chamber for a 3-hr heroin-seeking test. The heroin-seeking test took place under the same conditions as the self-administration training, except pressing on the active lever did not result in a heroin infusion, i.e., rats were tested under extinction conditions.

Locomotor activity test. Three days following the blood collection rats completed a locomotor activity test. Rats were placed into a locomotor activity chamber (39.0 x 42.0 x 50.0 cm) for a one-hour test session. The chamber is composed of a 16 x 16 infrared photocells matrix (Coulbourn Instruments). The chamber recorded the total distance traveled by rats. This test was to ensure that, if an effect of corticosterone is determined, the observed effect was not due to differences in general locomotor activity.

Tissue Collection. Immediately following the locomotor activity test, the animals were humanely euthanized. This was done with an overdose injection of Euthanyl (sodium pentobarbital). Rats were then intracardially perfused with phosphate-buffer-saline (1X PBS), paraformaldehyde (4%). Brains were extracted and postfixed for 24 hours, dehydrated with 30% sucrose at 4°C for 48 hours then stored in -80°C.

Statistical Analyses.

The critical threshold for statistically significant results was set at $p < .05$. The number of active and inactive lever presses made during the heroin-seeking test was analyzed separately using a one-way ANOVA to compare the mean lever presses between the 3 treatment groups. Total distance traveled during the locomotor test was analyzed using a two-way repeated-measures ANOVA with the between-subjects' factor of treatment group and the within-subjects' factor of time (6 x 10-minute intervals).

Data Integrity.

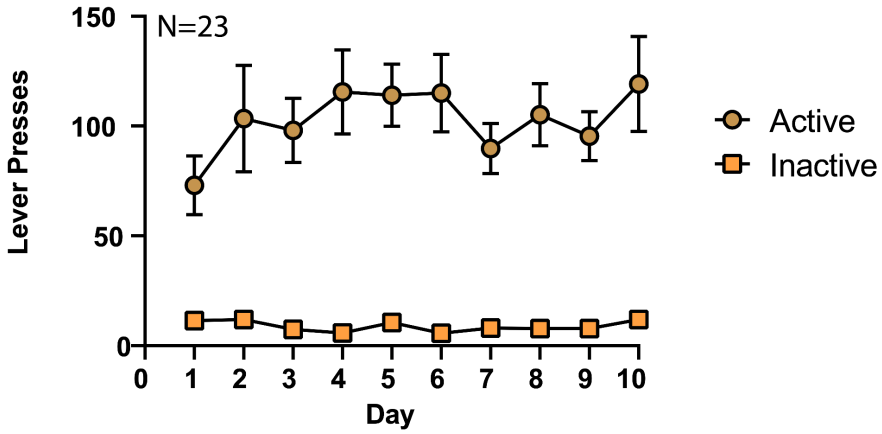
Six rats were removed due to failure to train, health issues, or catheter leakage. One rat was considered an outlier due to an extreme number of active lever responses made during the heroin self-administration training period (>2.5 *SD* above group average). Ten rats were removed from the statistical analysis due to technical issues (i.e., extreme noise and vibrations) where we believe that noise from the construction of a new building affected the behavior of the rats during the test. Therefore, the final analysis included 23 rats across the three experimental conditions: water (n = 8), vehicle (n = 8), CORT (n = 7).

Assumptions for a one-way ANOVA include normality, independence, and homogeneity of variance. None of these assumptions were violated following the inspection of the data. Assumptions for a two-way repeated-measures ANOVA include normality, independence, and sphericity. These assumptions were also not violated following the inspection of the data.

All rats learned to dissociate between the active and inactive lever and demonstrated reliable heroin self-administration. This learning is evident by the robust increase in active lever presses (see Figure 1) and a consistently low amount of inactive lever presses (see Figure 1) throughout the heroin self-administration training. Group Means and SEM for the number of active lever responses, inactive lever responses, infusions, and bodyweight during the last five days of heroin self-administration training, for each treatment group is presented in Table 1. There were no statistically significant differences between the different treatment groups on any of these variables.

Figure 1

Active and inactive lever pressing during self-administration training



Note. The line graph shows a robust increase in active lever responses throughout the 10 days of heroin self-administration. Error bars represent standard error of the mean.

Table 1

Heroin Self-Administration Training

	Infusions	Active Lever	Inactive Lever	Bodyweight
Water	36.10 ± 5.40	89.60 ± 17.47	10.18 ± 1.81	351.95 ±6.45
Vehicle	38.60 ± 4.32	108.43 ± 14.65	8.40 ± 1.53	346.48 ± 4.46
CORT	36.00 ± 6.76	118.43 ± 29.34	7.00 ± 1.61	341.86 ± 8.15

Results

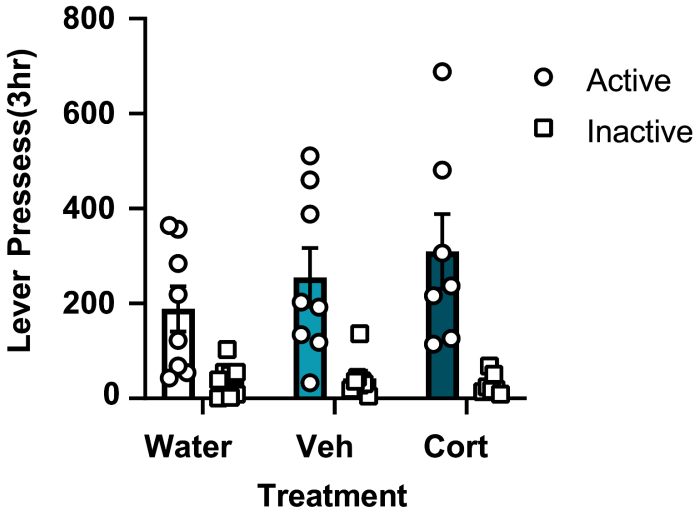
Heroin Seeking Test

A one-way ANOVA was conducted to determine if chronic corticosterone administration during the abstinence period (i.e., 15 days) had an impact on active and inactive lever responding during the heroin seeking test when compared to the control groups. The one-way ANOVA revealed no statistically significant differences between treatment groups for both active lever responses (F (2, 20) = .92, p = .42, η^2 = 0.03; see Figure 2) and inactive lever responses (F (2,20) = 0.31, p = .73, η^2 = .01; see Figure 2). The model estimates for both one-way ANOVA's are presented in Appendix A. This suggests that all rats pressed the active and inactive lever at a similar rate, regardless of

the treatment that was administered. These results indicate that the hypothesis was not supported indicating that chronic corticosterone administration does not appear to increase heroin seeking following a period of prolonged abstinence.

Figure 2

Heroin seeking test after forced abstinence and corticosterone treatment



Note. The bar graph shows that the number of active lever responses during the heroin seeking slightly increased with corticosterone treatment, however this increase failed to reach statistical significance ($F_{(2, 20)} = 0.92$, $p = .42$, $\eta^2 = 0.03$). Responses on the inactive lever, as expected, did not differ across treatment conditions ($F_{(2,20)} = 0.31$, $p = .73$, $\eta^2 = 0.01$). Error bars represent standard error of the mean.

Although no statistically significant effects were detected, visual inspection of the data suggests a pattern of increased responses. To further analyze this relation, Cohen's d was calculated to assess the magnitude of the differences in active lever responses between the corticosterone treatment condition and the control treatment conditions: CORT vs. water, $d = 0.75$, CORT vs. vehicle $d = 0.30$, and vehicle vs. water $d = 0.45$. These effect sizes are relatively low compared to the effect sizes that are usually observed for the food restriction impact, revealing that although CORT increased active lever responses, this increase is largely attributed to the fact that the vehicle does have an effect on heroin seeking.

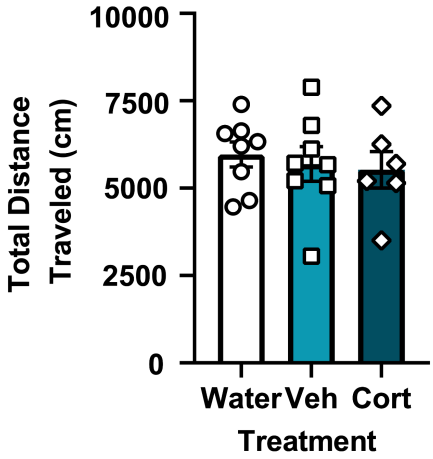
Locomotor Activity Test

Datum for the locomotor activity test were lost for one rat. Therefore, the final analysis included 22 rats across the three treatment conditions: water ($n = 8$), vehicle ($n = 8$), CORT ($n = 6$). The repeated- measures ANOVA revealed no statistically significant difference in total distance traveled between the treatment conditions (F (2,19) = 0.42, $p = .67$, $\eta^2 = 0.002$; see Figure 3), suggesting that chronic CORT administration did not have an impact on locomotor activity. There was a statistically significant main effect of time (F (5,95) = 108.2, $p < .001$, $\eta^2 = 0.85$), but no statistically significant time x treatment interaction (F (10,95) = 0.78, $p < .64$, $\eta^2 = 0.07$). These results suggest that as the locomotor activity test progressed, all rats, regardless of their treatment

condition, gradually decreased their locomotor activity.

Figure 3

Total locomotor activity following corticosterone treatment



Note. The bar graph shows the total distance traveled. As expected, locomotor activity did not differ across the treatment conditions ($F_{(2,19)} = 0.42$, $p = .67$, $\eta^2 = 0.002$). Error bars represent standard error of the mean.

Discussion

The overall goal of this experiment was to assess the effect of chronic exogenous administration of corticosterone during abstinence on heroin seeking. We predicted that chronic corticosterone administration would increase heroin seeking compared to control groups. The results suggest that chronic exogenous corticosterone administration did not increase heroin-seeking following 15 days of abstinence, compared to vehicle-treated rats. Therefore, the main hypothesis that chronic corticosterone manipulation would augment heroin seeking was not supported. This was substantiated by the non-significant differences in lever responding between the treatment groups during the heroin seeking test. Corticosterone was administered orally, wherein the organic mechanism of the HPA axis was interfered with. When researching stress, we target this axis because its activation is linked with the occurrence of a stressful event [20]. The HPA axis organically releases corticosterone, once a stimulus triggers the hypothalamus, leading to the release of corticosterone [22].

Due to the exogenous administration of corticosterone at higher concentrations than peak plasma concentrations, there may have been an overwhelming effect to the system. This could have caused an attenuation of corticosterone release as well as other stress-related systems, which may have led to an inhibitory effect on the stress experienced by rats. Therefore, the rats in the corticosterone treatment group were possibly experiencing similar amounts of stress in relation to the other treatment conditions. Due to the possibility that all treatment groups were experiencing similar stress levels, this may have led them to have a similar amount of lever presses during the heroin seeking test. It is believed that heroin seeking increases following exposure to chronic food restriction due to the stress experienced by the rats [10]. Therefore, if the three treatment conditions experienced similar amounts of stress, there would be no reason to believe that there would

be differences in heroin seeking behaviours. When the HPA axis is stimulated the first neurohormone to be released is CRF, this cascade ends with the release of corticosterone from the adrenal glands [19]. A study by Shalev and colleagues demonstrated that CRF and not corticosterone was critically involved in the augmentation of heroin seeking following acute food deprivation [23]. Specifically, in this study when a CRF antagonist was administered to rats this attenuated subsequent heroin seeking. This effect was dose-dependent and resulted in a significant decrease in active lever responses compared to control. However, when rats had their adrenal glands surgically removed, a similar attenuation of heroin seeking following CRF administration was not observed. Therefore, due to the removal of adrenal glands and consequently no corticosterone release, this supports the role of CRF and not corticosterone when examining heroin seeking following acute food deprivation. We suspect that a similar effect would occur if replicated with animals that were chronically food-restricted, as opposed to food-deprived.

Another possibility for the lack of effect of corticosterone on heroin seeking is the difference in route of administration. More specifically, we administered corticosterone systemically, but local administration is an alternative. Research by Graf and colleagues demonstrated that animals injected with corticosterone directly into the nucleus accumbens, followed by cocaine administration showed a significant increase in cocaine seeking behaviour [24]. Therefore, this raises the possibility that local and not systemic administration results in an effect in drug seeking behavior. However, this research was conducted with cocaine, which has very different molecular effects compared to heroin. Therefore, local administration would need to be verified with heroin to validate the effect of corticosterone local administration. Thereby providing greater insight into the role that corticosterone may play in the alteration of heroin seeking following a period of prolonged abstinence.

This study is not without limitations. Firstly, because corticosterone was administered via the drinking water, we were unable to control the amount of volume consumed by each experimental animal. Therefore, the level of corticosterone varied among experimental animals. Secondly, as previously mentioned corticosterone is a stress-related hormone, secreted from the adrenal glands upon activation of the HPA axis. However, we exogenously administered corticosterone thereby interfering with the HPA mechanism. Rats were not exposed to a stressor. Lastly, ethanol was present in solution at a 2% concentration for both our vehicle and corticosterone treatment conditions. The consumption of ethanol may have influenced the heroin seeking behaviours of rats which may have had an impact on the results obtained in this study.

Despite these limitations, the results from this study provide us with a greater understanding into the role that corticosterone plays in the augmentation of heroin seeking following a prolonged period of abstinence. Chronic food restriction during abstinence in heroin trained rats induces increased heroin seeking, this effect was hypothesized to be mediated by elevated levels of corticosterone. However, we have indication that chronic exogenous corticosterone administration during an abstinence phase does not have a significant effect on rat's subsequent heroin seeking behaviours.

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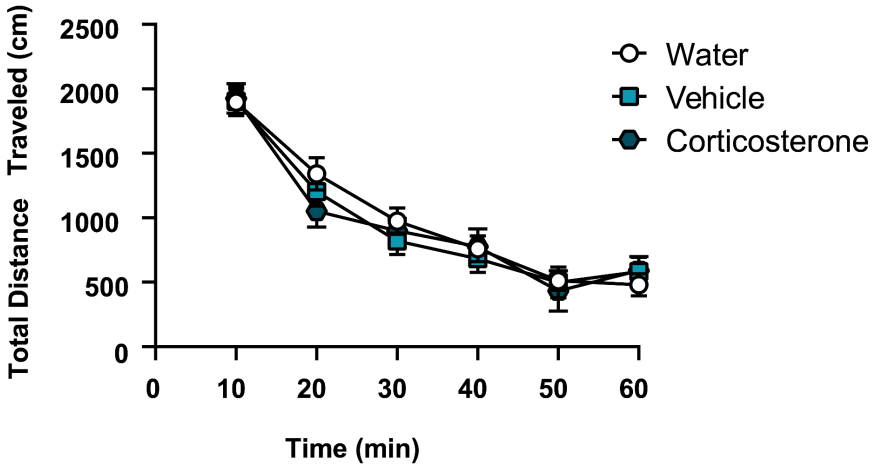
Appendix A

Locomotor activity across time following corticosterone treatment



Note. As expected, the locomotor activity across time decreased ($F(5, 95) = 108.2$, $p < .001$, $\eta^2 = 0.85$). There was not a significant difference across the treatment groups across time ($F(10, 95) = 0.78$, $p < .64$, $\eta^2 = 0.07$).