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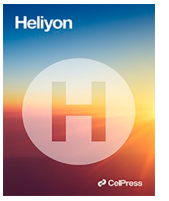

The cocaine compulsion zone theory explains the reinstatement of lever pressing behavior in rats in response to a single cocaine dose

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ABSTRACT

A single non-contingent dose of cocaine reinstates extinguished lever pressing behavior in rats trained to self-administer cocaine. This represents a model of relapse in cocaine use disorder and the number of lever presses has been the standard measure. Lever pressing behavior during self-administration occurs only when cocaine levels are below the satiety threshold and above the remission/priming threshold, a range termed the compulsion zone. Calculated cocaine levels at the time of each lever press during an FR1 self-administration session and following a single non-contingent dose of cocaine were compared. The mean latency to lever pressing behavior was dose dependent and ranged from 1 to 11 min after cocaine doses of 2 or 12 μmol/kg, respectively. This is consistent with higher cocaine doses producing levels above satiety threshold that take more time to fall back to that threshold. The level of cocaine when lever pressing occurred was similar whether cocaine was self-administered or after a single dose of cocaine. The number of lever presses after a single cocaine dose was variable and poorly dose dependent. The latency to the start of lever pressing behavior is a more reliable dependent measure than the number of lever presses. In addition, lever pressing behavior occurs only when cocaine levels are within the compulsion zone. The compulsion zone theory not only explains maintained cocaine self-administration behavior, but also explains the reinstatement of lever pressing behavior in response to a single non-contingent cocaine dose.

1. Introduction

Lever pressing behavior in rats [1,2] and monkeys [3] trained to self-administer cocaine extinguishes shortly after access to cocaine is terminated. However, lever pressing behavior is reinstated by the non-contingent administration of a single dose of cocaine [1–4]. This cocaine-induced reinstatement has been suggested to represent a model of persistent relapse in humans with cocaine use disorder [1–4]. The number of lever presses following the administration of a priming dose of cocaine is the established measure of the relapse event [5]. As higher cocaine doses tend to elicit a larger number of lever presses and for a longer duration it is assumed that this number reflects the reinforcing efficacy of cocaine [1]. Similarly, the number of lever presses has been the standard measure of reinstatement in rats and monkeys following an injection of several drug classes including stimulants, opioids, and alcohol [6–11].

Another approach to investigating cocaine-induced relapse is to gradually increase the cocaine level in the body by administering increasing micro doses of cocaine non-contingently to rats that are trained to self-administer cocaine but retain no cocaine from previous sessions. This titration of cocaine levels measures the minimum concentration of cocaine that reinstates self-administration

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behavior and is termed the priming threshold [12,13]. If cocaine levels continue to increase non-contingently after priming occurs, lever pressing continues until cocaine reaches a level termed the satiety threshold [13–15]. Above this level lever pressing rarely occurs. When cocaine injections are terminated and cocaine levels fall, lever pressing resumes when cocaine levels again reach the satiety threshold and continue until cocaine levels fall back to the priming/remission threshold [13]. The range of cocaine levels where lever pressing is observed is termed the compulsion zone, and this lever pressing behavior as cocaine concentrations fall through the compulsion zone appears similar to the lever pressing behavior observed after a single injection of cocaine [1].

Methods that titrate cocaine levels upward [12] from below the compulsion zone and the single cocaine dose reinstatement method [1] that has doses of cocaine that fall to and through the compulsion zone appear to provide complimentary information about the priming effect of cocaine. We investigated whether the non-contingent single cocaine dose reinstatement paradigm can be explained in terms of the compulsion zone theory [13] across a range of cocaine doses in rats trained to self-administer cocaine.

2. Results

2.1. Maintained cocaine self-administration

Rats exhibited maintained cocaine self-administered on the FR1 schedule in FR1 self-administration sessions performed once a week. Inter-injection intervals were regular at both unit doses (Fig. 1A). Inter-injection intervals were smaller at 0.3 μmol/kg with a mean of 0.89 min, and larger at 3 μmol/kg cocaine dose with a mean of 7.4 min (Table 1). Correspondingly, the rate of self-administration was 65 injections per hour at 0.3 μmol/kg and decreased to 9 per hour when the cocaine unit dose was switched to 3 μmol/kg. During unloading when access to cocaine was terminated and lever presses did not result in a cocaine injection, the rate of lever pressing was initially high. After approximately 20–40 min lever pressing slowed and then stopped.

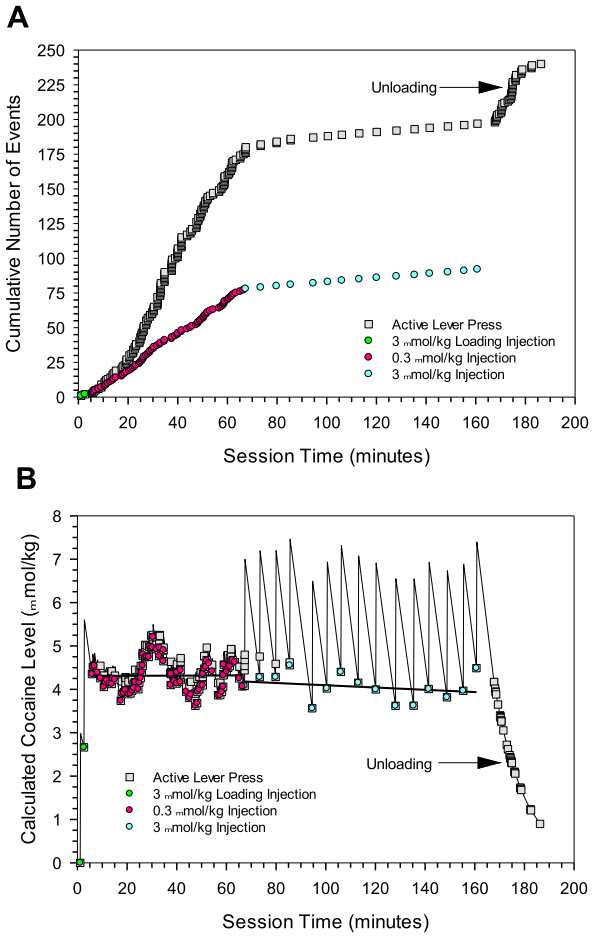

Fig. 1. Cumulative record of events (A) and calculated level of cocaine at the time of each event (B) in representative FR1 cocaine self-administration session. Each symbol represents an event. The injections were two initial self-administeredloading doses (3 μmol/kg), then rats self-administered two unit doses (0.3 (75 injections) then 3 μmol/kg (15 injections)). All lever presses on the active lever are also shown. After access to cocaine was terminated, active lever presses had no consequence but were recorded until lever pressing stopped.

Table 1
Effect of the unit dose of cocaine on the inter-injection intervals and cocaine level at the time of injection as well as the cocaine level at the time of the first lever press during when access to cocaine was terminated in FR1 cocaine self-administration sessions. Values represent the mean and standard error of the mean (from 7 rats) These data are from sessions conducted as shown in the representative FR1 self-administration session in Fig. 1.

	Inter-Injection Interval (minutes)	Calculated Cocaine Level (μmol/kg)
Injections (0.3 μmol/kg cocaine unit dose)	0.89 ± 0.06	4.04 ± 0.27
Injections (3 μmol/kg cocaine unit dose)	7.4 ± 0.36	3.75 ± 0.23
First lever press at Unloading (when access to cocaine was terminated)	N/A	3.83 ± 0.21

Fig. 1B shows the same data as Fig. 1A, but in terms of calculated cocaine level at the time of each event during the FR1 cocaine self-administration session. Despite the more than 7-fold higher rate of self-administration at the 0.3 compared to the 3.0 μmol/kg unit doses (Fig. 1A), the calculated cocaine levels at the time of injection were similar across these unit doses and averaged about 4 μmol/kg. Lever pressing behavior persisted during unloading and the calculated cocaine level at the onset of lever pressing during unloading was similar to the level at the time of injections during maintained self-administration. Lever pressing during unloading ceased when the calculated cocaine level decreased to about 0.7 μmol/kg.

Table 1 shows the average inter-injection interval at 0.3 μmol/kg and 3 μmol/kg cocaine dose from all FR1 cocaine self-administration sessions. Calculated cocaine level at the time of injection at each unit dose, and at the first lever press when access

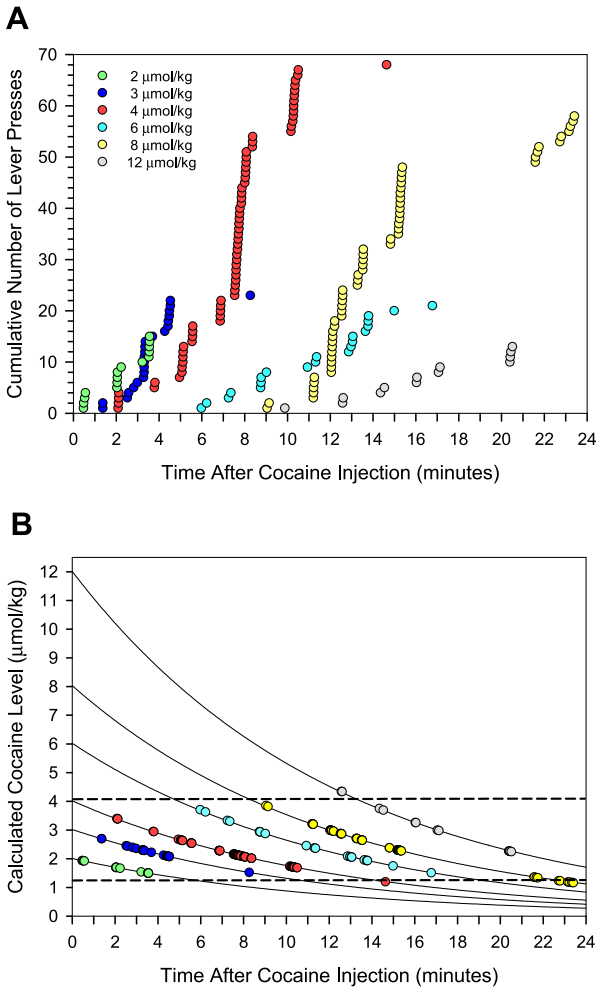

Fig. 2. Representative single cocaine dose reinstatement sessions from the same rat showing each non-contingent single cocaine dose administered. Cumulative number of lever presses on the active lever (A) that occurred after completion of the injection until lever pressing stopped, and corresponding calculated cocaine levels (B) are shown. Each symbol represents a lever press and the descending curves in B represent calculated cocaine level over time for each cocaine dose. The upper and lower dotted horizontal lines represent the approximate cocaine level at the onset and cessation of lever pressing behavior respectively. 2, 3, 4, 6, 8, and 12 μmol/kg is equivalent to 0.7, 1.0, 1.4, 2.0, 2.7, and 4.1 mg/kg cocaine HCl respectively.

to cocaine was terminated is also shown. Inter-injection intervals differed between the two doses, while the cocaine level at the time of injection was similar. The cocaine level at the first lever press at unloading was similar to the cocaine level during at the time of cocaine self-administration. Welch's *t*-test showed that the inter-injection intervals at the two cocaine unit doses were significantly different from each other, t (6.318) = −17.94. P < 0.001. One Way Analysis of Variance showed that the cocaine level during self-administration at both unit doses, and during the first unloading lever press did not have significant difference, F (2,18) = 0.403, P = 0.674.

2.2. Latency to reinstatement of lever pressing behavior after single cocaine injection is dependent on the cocaine dose and calculated cocaine level

Lever pressing behavior was observed after administration of each single dose in the representative reinstatement sessions (Fig. 2). The presence of a delay in the onset of lever pressing activity was observed for each dose, and this delay was dose dependent. The rat began lever pressing as early as 1 min after injection of 2 μmol/kg cocaine, the lowest dose, and it took up to 11 min for the rat to start lever pressing after the injection of 12 μmol/kg cocaine, the highest dose (Fig. 2A). The number of lever presses performed by the rat after injection of cocaine was variable and did not appear to be dose-dependent.

Fig. 2B represents the same data as Fig. 2A, but in terms of calculated cocaine level at each lever press shown in Fig. 2A. The cocaine level at the onset of lever pressing behavior was similar at across all doses, especially the high doses whose peak levels were above the cocaine levels maintained during FR1 cocaine self-administration sessions. It appears that the dose-dependent delay in onset of lever pressing behavior was a consequence of this observation.

The calculated cocaine level at the last lever press was also similar across all doses. The similarity in calculated cocaine levels at the onset of lever pressing behavior was consistent with levels maintained at the time of injection during FR1 cocaine self-administration (Fig. 1B, and Table 1). The calculated cocaine level at the time of the last lever press was consistent with the cocaine level at the last lever press when access to cocaine was terminated during unloading phase of FR1 cocaine self-administration (Fig. 1B).

2.3. Latency to reinstatement of lever pressing activity is proportional to cocaine dose

The average time to commencement of lever pressing activity (latency) after an injection of cocaine was proportional to the dose (Fig. 3). The standard error of the mean was relatively small for each dose, so the observed latency to lever pressing was consistent among this population of rats. The dose dependency was clear for 6 μmol/kg, 8 μmol/kg and 12 μmol/kg doses of cocaine. However, latency was similar for the 2 μmol/kg, 3 μmol/kg and 4 μmol/kg cocaine doses, and the rats pressed the lever within 2 min after being injected these lower cocaine doses. One Way Analysis of Variance showed a statistically significant difference in the latency to lever pressing behavior across all the doses, F (5,36) = 47.474, P = < 0.001.

2.4. Duration of lever pressing activity following a single cocaine dose

The average time for rats to exhibit cocaine-induced lever pressing behavior (duration) was longer for 6 μmol/kg, 8 μmol/kg and 12 μmol/kg and was lower for the 2 μmol/kg, 3 μmol/kg and 4 μmol/kg cocaine doses (Fig. 4). This duration of lever pressing activity was similar across lower doses (2 μmol/kg, 3 μmol/kg and 4 μmol/kg). Kruskal-Wallis One Way Analysis of Variance on Ranks showed no statistically significant difference between the three lower cocaine doses, H (2) = 0.364, P = 0.834. Likewise, duration of lever pressing activity was similar across the higher doses (6 μmol/kg, 8 μmol/kg and 12 μmol/kg) as confirmed by the results of Kruskal-

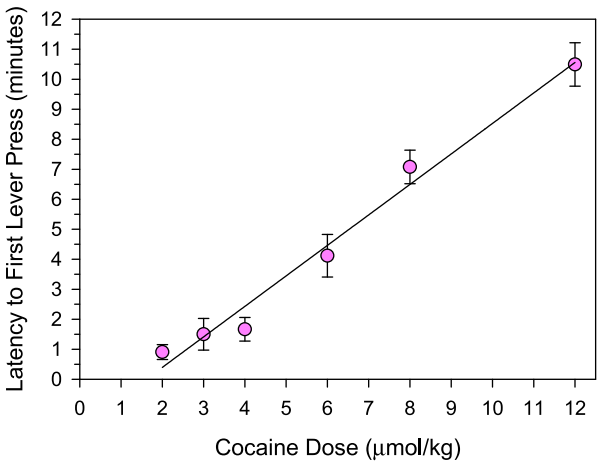

Fig. 3. The time taken to onset of lever pressing behavior (latency) after the injection of a single i.v dose of cocaine. Each symbol represents the mean latency and the bars represent the standard error of the mean. A linear regression model ($r^2 = 0.98$) was applied to the data.

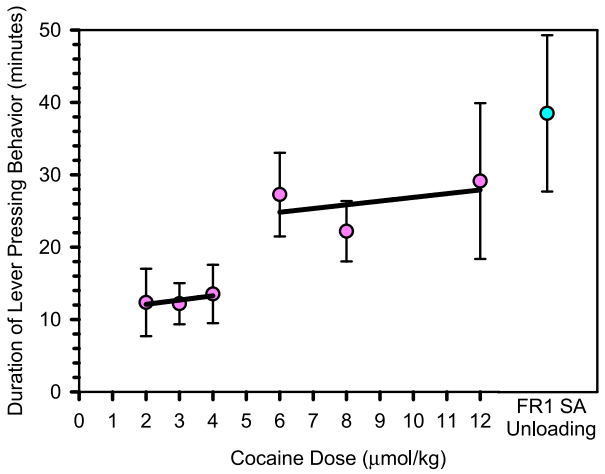

Fig. 4. The duration of maintained lever pressing behavior after the injection of a single i.v dose of cocaine (pink circles), and during unloading phase of standard FR1 cocaine self-administration session or FR1 SA Unloading (cyan circle). Each data point represents the mean duration and error bars represent the standard error of the mean for a cocaine dose or FR1 SA Unloading.

Wallis One Way Analysis of Variance on Ranks showing no statistically significant difference between the three higher cocaine doses, H (2) = 0.319, P = 0.853. However, the duration of lever pressing across these two dose ranges was dissimilar. While the average duration was 13.1 min for the lower doses, it was doubled to 26.2 min for the higher doses. The duration of lever pressing at unloading after maintained cocaine self-administration (FR1 SA Unloading) was longer at 38.5 min on average, but, Kruskal-Wallis One Way Analysis of Variance on Ranks showed that it was not significantly different than the duration of lever pressing at the higher cocaine

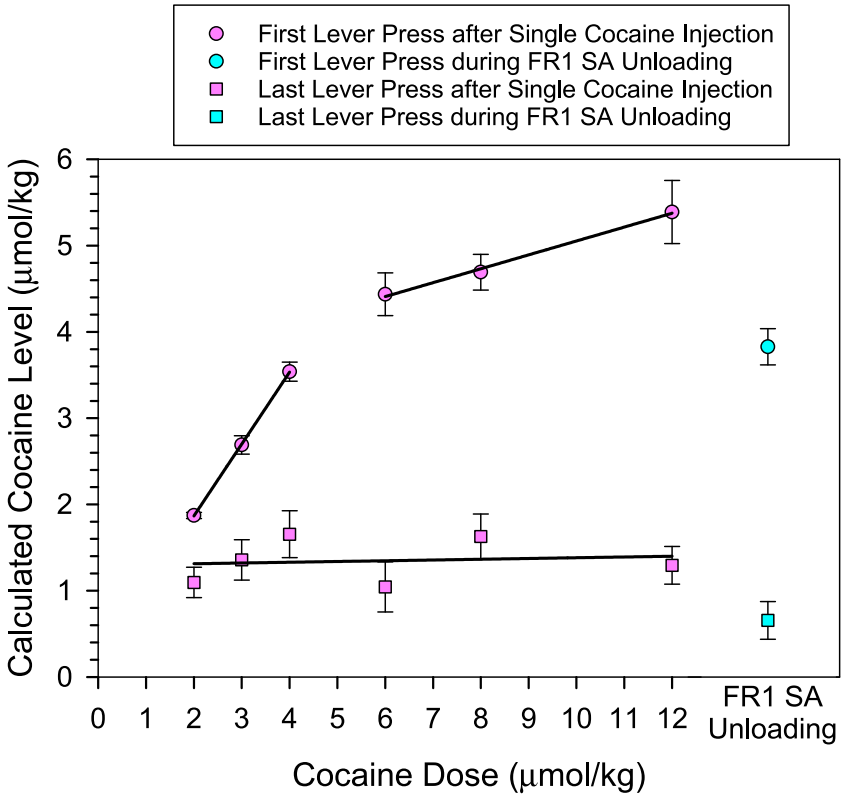

Fig. 5. Calculated cocaine level at the first and last cocaine induced lever press (pink circles and pink squares respectively) after the injection of a single i.v dose of cocaine; and the first and last lever press (cyan circle and cyan square respectively) during unloading phase of standard FR1 cocaine self-administration session or FR1 SA Unloading. Each data point represents the mean calculated cocaine level and the error bars represent the standard error of the mean for the corresponding lever press for a dose or FR1 SA Unloading.

doses (6 μmol/kg, 8 μmol/kg and 12 μmol/kg), H (2) = 1.075, P = 0.783.

2.5. Differential effect of dose on calculated cocaine level at first and last lever press

The average calculated cocaine level at the first and last lever press after administration of a single cocaine dose is shown in Fig. 5. The figure also shows the average calculated cocaine level at the first and the last lever press during unloading after the standard FR1 cocaine self-administration sessions (FR1 SA Unloading). Calculated cocaine levels at the last lever press were comparable across the entire range of the single doses of cocaine, and the results of One-Way Analysis of Variance showed that these values did not have a statistically significant difference, F (5,36) = 1.107, p = 0.374. Additionally, analysis also showed that calculated cocaine levels at the last lever press after injection of all cocaine doses and at the last lever press during FR1 SA Unloading also did not have a statistically significant difference, F (6,42) = 2.121, P = 0.071.

In contrast, the calculated cocaine level at the first lever press differed across the range of single doses, and Kruskal-Wallis One-Way Analysis of Variance showed that there was a statistically significant difference H (5) = 35.447, P = < 0.001 among the doses. As seen based on the two different slopes, calculated cocaine level at first lever press were different between the lower doses (2 μmol/kg, 3 μmol/kg and 4 μmol/kg) and higher doses (6 μmol/kg, 8 μmol/kg and 12 μmol/kg). The average cocaine level at the first lever press was 2.7 μmol/kg for the lower doses and 4.8 μmol/kg for the higher cocaine doses. However, One-Way Analysis of Variance showed no statistically significant difference between the cocaine level at first lever press following a single injection 6 μmol/kg, 8 μmol/kg and 12 μmol/kg cocaine doses (F (2,18) = 3.067, P = 0.071).

The average cocaine level at first lever press during FR1 SA Unloading was comparable with the level at first lever press after administration of the higher single cocaine doses. The average cocaine level at the first lever press for higher doses and FR1 SA Unloading was 4.6 μmol/kg. One Way Analysis of Variance showed a statistically significant difference in calculated cocaine level at the first lever press for the 6 μmol/kg, 8 μmol/kg and 12 μmol/kg cocaine doses and FR1 SA Unloading. Post Hoc Test using the Holm-Sidak method showed that there was no statistically significant difference in cocaine level at the first lever press between FR1 SA Unloading and 6 μmol/kg and 8 μmol/kg dose (P > 0.05). Though, there was a significant difference between FR1 SA Unloading and 12 μmol/kg cocaine dose (P < 0.05).

2.6. Number of lever presses is highly variable

The number of lever presses during maintained lever pressing behavior after administration of a single cocaine dose was highly variable (Fig. 6). Kruskal-Wallis One Way Analysis of Variance on Ranks showed no statistically significant difference between the number of lever presses at the cocaine doses, H (5) = 6.896, P = 0.229. The number of lever presses during unloading after standard cocaine self-administration (FR1 SA Unloading) was also variable (Fig. 6). Another Kruskal-Wallis One Way Analysis of Variance on Ranks showed that number of lever presses after injection of all doses were not significantly different than the number of lever presses during FR1 SA Unloading, H (6) = 9.795, P = 0.134. The error bars indicating standard error of the mean imply that rate of lever pressing might be highly variable.

3. Discussion

Cocaine self-administration behavior in rats on an FR1 schedule of cocaine delivery was regular at a given unit dose and the inter-injection intervals were proportional to the unit dose, with a 10-fold increase in unit dose producing an 8-fold increase in the inter-

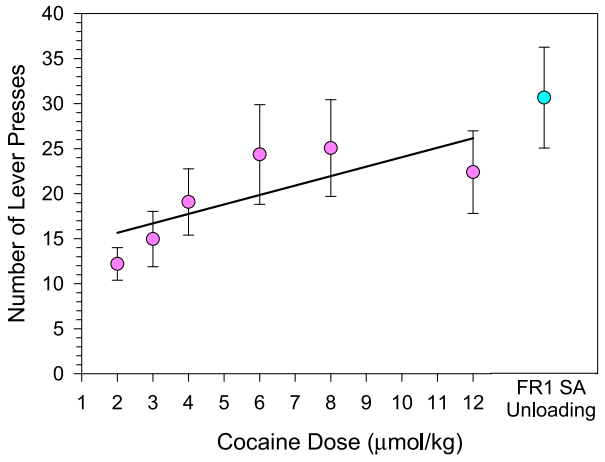

Fig. 6. The number of lever presses after the injection of a single i.v dose of cocaine (pink circles) and during unloading phase of standard FR1 cocaine self-administration session or FR1 SA Unloading (cyan circle). Each data point represents the mean number of lever presses and error bars represent the standard error of the mean for a cocaine dose or FR1 SA Unloading.

injection interval. In contrast, the calculated cocaine concentration at the time of each injection was similar across these different unit doses. These observations are consistent with the satiety threshold model of maintained cocaine self-administration behavior [14], and indicate that the satiety threshold was constant during a FR1 cocaine self-administration session and independent of the cocaine unit dose. After termination of access to cocaine, the level at which unloading begins was not different than the satiety threshold observed during maintained self-administration. Once lever pressing was initiated, the rate was more rapid than the rate of self-administration at even the lowest unit dose and persisted for approximately 20 min before slowing down and eventually stopping when cocaine levels fell to or below the remission threshold. This lever pressing behavior is consistent with the compulsion zone theory of cocaine self-administration [13], which states that lever pressing behavior is induced by cocaine only when cocaine levels are below the satiety threshold and above the priming/remission threshold.

The reinstatement of lever pressing behavior in rats trained to self-administer cocaine following a single non-contingent dose of cocaine is a well-established phenomenon [1,5]. The present study confirms this phenomenon. In the previous studies the number of lever presses was reported to be proportional to the dose and was the dependent measure used [1,5]. However, in the present study the total number of lever presses following a single dose of cocaine was weakly correlated with dose and the variability between rats and between reinstatement sessions was relatively high. Instead, the most prominent and reproducible observation was the dose-dependent latency to the reinstatement of lever pressing behavior after the injection of cocaine. Although this phenomenon was reported in previous studies and was suggested to be similar to the increased inter-injection interval during self-administration of higher doses of cocaine [1], it was never recognized that this latency represents a useful measure. Our current study is in agreement with this interpretation and, the dose-dependent latency is consistent with the higher doses of cocaine being above the satiety threshold, thereby inhibiting lever pressing. Once the cocaine level falls to the satiety threshold lever pressing is induced. The higher doses take longer to fall back to the satiety threshold, hence the increase in latency to lever pressing at higher doses.

Once levels of cocaine fell to the satiety threshold lever pressing began and continued while cocaine levels transited the compulsion zone. The duration of lever pressing behavior was similar across the higher doses and these durations were similar to the duration of lever pressing behavior after the termination of access to cocaine during the FR1 self-administration studies. These observations are consistent with the compulsion zone being relatively constant irrespective of whether a single non-contingent dose of cocaine was injected or whether cocaine was self-administered. The shorter duration of lever pressing after a single lower dose of cocaine is consistent with these lower doses resulting in cocaine levels that are already within the compulsion zone. Therefore, these lower concentrations do not transit the entire compulsion zone and reach the remission threshold in a shorter time.

The similarity of the levels of cocaine at which the last lever press occurred across the wide range of doses, and following the termination of access to cocaine after self-administration, indicates that the remission threshold does not vary in these rats irrespective of the length of time that cocaine was in the compulsion zone.

It would be expected that the level of cocaine at the time of the resumption of lever pressing would correspond to the satiety threshold and this would be constant across the higher unit doses that result in a cocaine level above the satiety threshold. However, the higher calculated level of cocaine at the resumption of lever presses was higher at the highest cocaine dose. It is possible that the highest dose of cocaine produced an increase in the satiety threshold. A number of pharmacological interventions have been documented to increase the satiety threshold including competitive dopamine receptor antagonists and an irreversible monoamine receptor antagonist [16–19]. Whether this single high dose of cocaine induces desensitization of dopamine receptors underlying the satiety threshold, thereby requiring a higher cocaine dose to induce the satiety response, will require further study.

A single dose of amphetamine [6,7,10], MDMA [9], heroin [8], morphine [6], and alcohol [11], reinstated lever pressing behavior in rats or monkeys trained to self-administer the relevant drug. Whether the compulsion zone theory is applicable to the self-administration of these different drugs and can explain the single dose reinstatement of lever pressing behavior is not established at present.

In summary, the resumption of lever pressing behavior in response to a single non-contingent dose of cocaine is similar to the lever pressing behavior following termination of access to cocaine following an FR1 cocaine self-administration session. Whether cocaine is delivered non-contingently as a single dose or contingently during maintained cocaine self-administration behavior, lever pressing only occurs when cocaine levels are within the compulsion zone.

4. Experimental procedure

4.1. Animals

Male Sprague Dawley rats (N = 7) initial weight 200–225 g and 350–550 g over the duration of studies from Envigo (Indianapolis) were housed individually on a 14/10 h light/dark cycle with food and water available ad libitum. All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and under a protocol approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

4.2. Surgery

Rats were surgically implanted with an indwelling catheter into the right jugular vein under isoflurane anesthesia. If re-catheterization was required, catheters were placed in the left jugular and then the femoral veins as needed throughout the study. Buprenorphine (0.03 mg/rat s.c.) was administered post-surgery for pain control and gentamycin (25 mg/rat s.c.) for three days was used to prevent infection following surgery. The catheter was flushed with heparin solution (100 units/mL in saline) once a day for the

first five days after surgery.

4.3. Cocaine self administration training

Beginning at least 5 days after surgery, rats were trained to self-administer cocaine HCl (provided by the National Institute on Drug Abuse) using a fixed ratio (FR1) schedule with a timeout period equal to the injection time or 5 s, whichever was longer. Rats were weighed and flushed with 0.5 mL of heparin solution (100 units/mL in saline) immediately prior to each self-administration session. Self-administration training sessions began from 9 to 10 a.m. Rats were placed in isolated chambers equipped with both an active and an inactive lever. The standard unit dose for training was 3 μmol/kg of cocaine HCl (40 μmol/mL in sterile saline with 1 unit heparin/mL solution). Presses on the active lever caused activation of the pump and resulted in injection. The rate of injection of the cocaine solution was 4 μL/s. A cue light illuminated for the duration of the timeout accompanied pump activation. Presses on the inactive lever had no consequences. Presses on the active lever during time out, and all presses on the inactive lever were recorded. Detailed protocol for cocaine self-administration training and the real time computation of cocaine levels can be found in Ref. [20]. Rats had access to cocaine for 3 h a day, five days a week. The training was considered complete when rats met the criterion for acquisition, that is inter-injection intervals did not systematically deviate from day to day for three consecutive training sessions. After acquisition of maintained self-administration at 3 μmol/kg of cocaine dose, rats were allowed to self-administer cocaine on FR1 schedule in two dose self-administration sessions. During the two dose self-administration sessions rats first received two priming injections of 3 μmol/kg cocaine dose. Next, they received a fixed number of injections of the first dose followed by a fixed number of injections of the second dose. Lastly, rats entered the extinction or unloading phase where active lever presses were recorded but had no consequence. The session was determined complete when 30 min had elapsed since the last active lever press that occurred during the unloading phase. The cocaine doses were 0.3 μmol/kg, 0.75 μmol/kg, 1.5 μmol/kg, 3 μmol/kg, 6 μmol/kg, 12 μmol/kg, and the fixed number of injections for each dose was 75, 50, 25, 15, 10, and 5 respectively. Each of these two dose self-administration sessions were repeated three times. The inter-injection intervals were measured and cocaine level at each lever press was calculated.

4.4. Single cocaine dose reinstatement training and schedule

Rats were switched to the Single Cocaine Dose Reinstatement experiment after successful cocaine self-administration training. Every Monday throughout the course of the study, rats were on a standard FR1 cocaine self-administration session (2 priming doses of 3 μmol/kg, 75 doses of 0.3 μmol/kg and 15 doses of 3 μmol/kg cocaine followed by unloading) to ensure that cocaine self-administration persisted and that the catheter was patent. Then, on Tuesday-Friday, rats were on a single cocaine dose reinstatement session and they were run on one reinstatement session in a day. For the single cocaine dose reinstatement sessions, the active lever was disabled so that once the rat was in the chamber, any and all lever presses had no consequences. Rats received only a single non-contingent cocaine injection that was delivered by the program. The injection occurred 1 min after the reinstatement session was started in cases where spontaneous lever presses on the active lever did not occur. If the rat performed spontaneous lever presses on the active lever when the reinstatement session started, the injection occurred 1 min after the most recent spontaneous lever press.

The non-contingent cocaine injection was one out of 2 μmol/kg, 3 μmol/kg, 4 μmol/kg, 6 μmol/kg, 8 μmol/kg, 12 μmol/kg, or the vehicle saline. Cocaine dose was determined by the pump activation time (4.1–43 s), and therefore the injection volume (0.019–0.17 mL), which varied depending on the dose and weight of the rat (377–574 g). The volume of vehicle injected was equivalent to the volume of a 12 μmol/kg cocaine dose. The dose to be administered on a day was chosen randomly. There were 3–5 times per rat for each cocaine dose. All active lever presses that occurred after the non-contingent injection of the cocaine dose were recorded, and had no consequences. Reinstatement sessions were ended when 30 min had elapsed since the last active lever press. Calculated cocaine levels at the time of each active lever press were also recorded.

4.5. Data analysis

The number of lever presses, inter-injection intervals, duration of unloading, and the calculated cocaine level at the time of active lever presses were collected from each standard FR1 cocaine self-administration session. Seven rats were run on FR1 self-administration sessions and there were 4–11, with a mean of 7 of these standard FR1 self-administration sessions per rat. Inter-injection intervals at 0.3 and 3 μmol/kg cocaine unit dose, and the average calculated cocaine level at the time of 0.3 and 3 μmol/kg cocaine unit dose injections were analyzed. Duration of unloading, calculated cocaine level at first unloading lever press, calculated cocaine level at last unloading lever press, and total number of lever presses during unloading was also analyzed. For each measure, the values from all the sessions from a rat were used to calculate a mean for the rat. Next, the mean values of the rats were used to calculate a final mean and standard error of the mean. The mean inter-injection intervals at 0.3 and 3 μmol/kg cocaine unit dose was compared using Welch's *t*-test. The mean values of the average calculated cocaine level at the time of 0.3 dose and 3 μmol/kg cocaine dose injections, and at first unloading lever press from each rat was compared using One Way Analysis of Variance.

The number of lever presses after injection, time to first lever press after the injection, duration of lever pressing behavior after injection, and calculated cocaine concentrations at active lever presses were collected from each single cocaine dose reinstatement session. Reinstatement sessions with three or less lever presses after the programmed injection were not used in data analysis. The number of reinstatement sessions for each cocaine unit dose 2 μmol/kg, 3 μmol/kg, 4 μmol/kg, 6 μmol/kg, 8 μmol/kg, and 12 μmol/kg was 23, 22, 22, 24, 30 and 25 respectively. Time to first lever press, duration of lever pressing behavior, calculated cocaine level at the first lever press, calculated cocaine level at the last lever press, and total number of lever presses after the single cocaine injection were

analyzed and graphed in scatter plots. For each measure, the values from all the reinstatement sessions of a cocaine dose from a rat were used to calculate a mean for the rat. Next, the mean values of the rats were used to calculate a final mean and standard error of the mean for each cocaine dose. The mean and standard error of the mean of the different measures for the cocaine doses were displayed in scatter plots.

The mean and standard error of the mean of the duration of unloading, calculated cocaine level at first unloading lever press, calculated cocaine level at last unloading lever press, and total number of lever presses during unloading from the standard cocaine self-administration procedure were compared with the corresponding measures from the single dose reinstatement procedure. The mentioned measures were included as data points in the scatter plots of duration of lever pressing behavior, calculated cocaine level at the first lever press, calculated cocaine level at the last lever press, and total number of lever presses after the single cocaine injection from the single dose reinstatement procedure.

Linear regression analysis was applied to the data, and analysis of variance was performed when appropriate.

Author contribution statement

Dakota B. Zinani: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Jhanvi N. Desai: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Andrew B. Norman: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

FR: Fixed ratio; FR1 SA Unloading: Unloading phase following standard cocaine self-administration at the fixed ratio 1 schedule.

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