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Differential Effect of Fixed Ratio Magnitude on the Rate of Lever-Pressing and Interinjection Intervals of Cocaine Self-Administration in Rats

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ABSTRACT

Background: Many features of self-administration behavior may be explained by reference to the properties of schedules of reinforcement. Schedules alter the probability of a behavior being reinforced and thereby increase, or decrease, the frequency of the behavior and fixed ratio (FR) magnitude reportedly alters the rate of responding to cocaine. A pharmacokinetic/pharmacodynamic interaction theory states that lever-pressing behavior is induced only when cocaine levels in the body are above the priming/remission threshold and below the satiety threshold—a range termed the compulsion zone. This theory successfully explains cocaine self-administration in rats on a progressive ratio and the FR1 schedule.
Objectives: To determine the effects of high FR magnitude on the rate of self-administration of cocaine and the rate of lever-pressing behavior when cocaine levels are within the compulsion zone.
Methods: Rats acquired cocaine self-administration on an FR1 schedule and then were switched to sessions that started with FR1 and then FR 5, 10, 20, or 50. An only FR1 session was run each week between FR1/FR50 sessions and then only FR1 sessions were conducted for several weeks.
Results: Interinjection intervals at a unit dose of 3 μmol/kg were regular at both FR1 and FR50 but were longer by the time required to complete the 50 presses. When responding by rats was maintained under an FR50 schedule of cocaine presentations, compared to baseline FR1 sessions, dramatic increases in the number of lever-presses were observed after access to cocaine was terminated, a previously unreported finding. However, lever-pressing occurred only when cocaine levels were in the compulsion zone, and this duration was unchanged. The increase in lever-pressing persisted for weeks. Interinjection intervals at FR1 were not altered after exposure to FR50.
Conclusions: Although previously considered key to understanding the regulation of cocaine self-administration behavior, FR magnitude simply increased interinjection intervals by the time required to complete 50 lever-presses. The dramatic increase in the rate of lever-pressing was caused by the high FR schedule rather than cocaine. The utility of the schedule-induced increase in the rate of lever-pressing is unclear. The compulsion zone theory provides a rational pharmacological basis for understanding cocaine self-administration behavior.

Introduction

The injection of various drugs is considered to serve as a reinforcer and this makes it possible to analyze drugs functionally in the same way as other events that can maintain behavior.[1] Many features of self-administration behavior may be explained by reference to the properties of schedules of reinforcement.[1,2] Schedules alter the probability of a behavior being reinforced and thereby increase, or decrease, the frequency of the behavior.[3]

It is long established that cocaine is a reinforcer in the self-administration paradigm in rats.[2,4,5] In their seminal study, Pickens and Thompson[2] investigated the effect of fixed ratio (FR) size on the cocaine self-administration paradigm. It was reported that

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proportionate increases in the frequency of responding were found as the FR magnitude was increased over the range 1, 5, 10, and 20. It was also reported that the response rate (lever-presses) varied inversely as a function of the cocaine unit dose and a striking characteristic of cocaine-reinforced behavior was reported to be long but regularly spaced pauses after reinforcement. These findings are universally observed in studies of drug self-administration using FR schedules.[6–9] When responding by rats was maintained under an FR1 schedule of cocaine presentations, these phenomena have been explained by a pharmacokinetic/pharmacodynamic interaction model that demonstrates that when the cocaine concentration is above the satiety threshold level, lever-pressing behavior is not observed[10] until cocaine is metabolized[11] and the level falls back to the satiety threshold. At higher unit doses, it takes longer for the higher cocaine levels to fall back to the satiety threshold, accounting for the longer interinjection intervals and their regularity. This is consistent with the first pharmacokinetic/pharmacodynamic theory of the regulation of amphetamine self-administration reported in rats.[8] Furthermore, it is established that cocaine induces lever-pressing behavior that occurs only when cocaine levels are below the satiety threshold and above the priming/remission threshold, a range of levels termed the compulsion zone.[12] It was previously reported that the compulsion zone accounts for the increases in breakpoint as a function of the cocaine unit dose using a progressive ratio (PR) schedule of cocaine self-administration in rats and that when the unit dose is equivalent to the width of the compulsion zone there is no further increase in breakpoint.[13] Furthermore, the maximum breakpoint in the PR schedule was the same as the number of presses after access to cocaine was terminated when cocaine was self-administered on an FR1 schedule.[14] Because the seminal work of Pickens and Thompson[2] cited the importance of FR size in regulating cocaine self-administration behavior, this present study revisited the effects of FR magnitude up to FR50 in light of our pharmacokinetic/pharmacodynamic interaction theory. We investigated the rate of cocaine self-administration behavior and the rate of lever-presses during maintained self-administration and after access to cocaine was terminated, the unloading (or extinction) phase of a session.

Methods

Animals

Male Sprague Dawley rats (n = 8) initial weight 200 to 225 g and 350 to 550 g over the duration of studies from Envigo (Indianapolis, Indiana) were housed individually on a 14/10 hour 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 and reported in accordance with ARRIVE guidelines.

Catheter implantation

Rats were surgically implanted with an indwelling catheter into the right jugular vein under isoflurane anesthesia. Throughout the course of the study catheter patency was tested by intravenous administration of short-acting barbiturate methohexital (Brevital sodium 6 mg/kg, 3-second injection), and the catheter was considered patent if it produced loss of righting reflex. If recatheterization was required, catheters were placed in the left jugular and then the femoral veins as needed throughout the study. Buprenorphine (0.03 mg/rat SC) was administered after surgery for pain control and gentamycin (25 mg/rat SC) for 3 days was used to prevent infection following surgery. The catheter was flushed with heparin solution (100 U/mL in saline) once a day for the first 5 days after surgery.

Cocaine self-administration training

Beginning at least 5 days after surgery, rats were trained for intravenous self-administration of cocaine hydrochloride (provided by the National Institute on Drug Abuse) using an FR1 schedule with a timeout period equal to the injection time or 5 seconds, whichever was longer. Rats were weighed and flushed with 0.5 mL heparin solution (100 U/mL in saline) immediately before each self-administration session. Self-administration sessions began from 9 to 10 AM. 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 cocaine hydrochloride (40 μmol/mL in 0.9% sterile saline with 1 U 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/sec. A cue light illuminated for the duration of the pump activation and during this time lever-presses were recorded but had no consequence (timeout). Presses on the inactive lever had no consequences. Rats had access to cocaine for approximately 3 hours a day, 5 days a week. The training was considered complete when rats met the criterion for acquisition; that is, interinjection intervals did not systematically deviate from day to day for 3 consecutive sessions. After acquisition of self-administration at the 3 μmol/kg cocaine dose, rats maintained responding on FR1 schedule of cocaine presentation over a range of unit doses during daily sessions. In these sessions, rats first self-administered 2 loading injections of 3 μmol/kg cocaine. Next, they self-administered a fixed number of injections of the first dose followed by a fixed number of injections of the second dose. Lastly, rats entered the unloading phase where access to cocaine was terminated and active lever-presses were recorded but had no consequence. These sessions were determined complete when 30 minutes had elapsed since the last active lever-press that occurred during unloading. 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. The injection time ranged from 0.5 to 42 seconds at the 0.3 and 12 μmol/kg dose, respectively, and the volume of solution injected ranged from 0.0021 to 0.17 mL depending on the unit dose and weight of the rat. The interinjection intervals were measured and the cocaine level at the time of each lever-press was estimated.

Estimation of cocaine levels

The cocaine level at the time of each lever-press was estimated as described previously.[15] Briefly, the cocaine level was computed every second of the session according to a 1-compartmental pharmacokinetic model that takes into account each cocaine injection amount and assuming first-order elimination with a half-life of 500 seconds.

FR1 cocaine self-administration

The sessions from cocaine self-administration training where rats maintained responding on FR1 schedule of cocaine presentation at the 0.3 μmol/kg and 3 μmol/kg unit dose (2 loading doses of 3 μmol/kg followed by 75 doses of 0.3 μmol/kg then 15 doses of 3 μmol/kg) are termed “FR1 baseline” sessions. In these sessions, after the 15th injection access to cocaine was terminated and unloading presses were recorded but had no consequence. There were 3 FR1 baseline sessions for each of the 8 rats.

Throughout the course of the study on high FR schedules, responding by the 8 rats was maintained under the same FR1 sched-

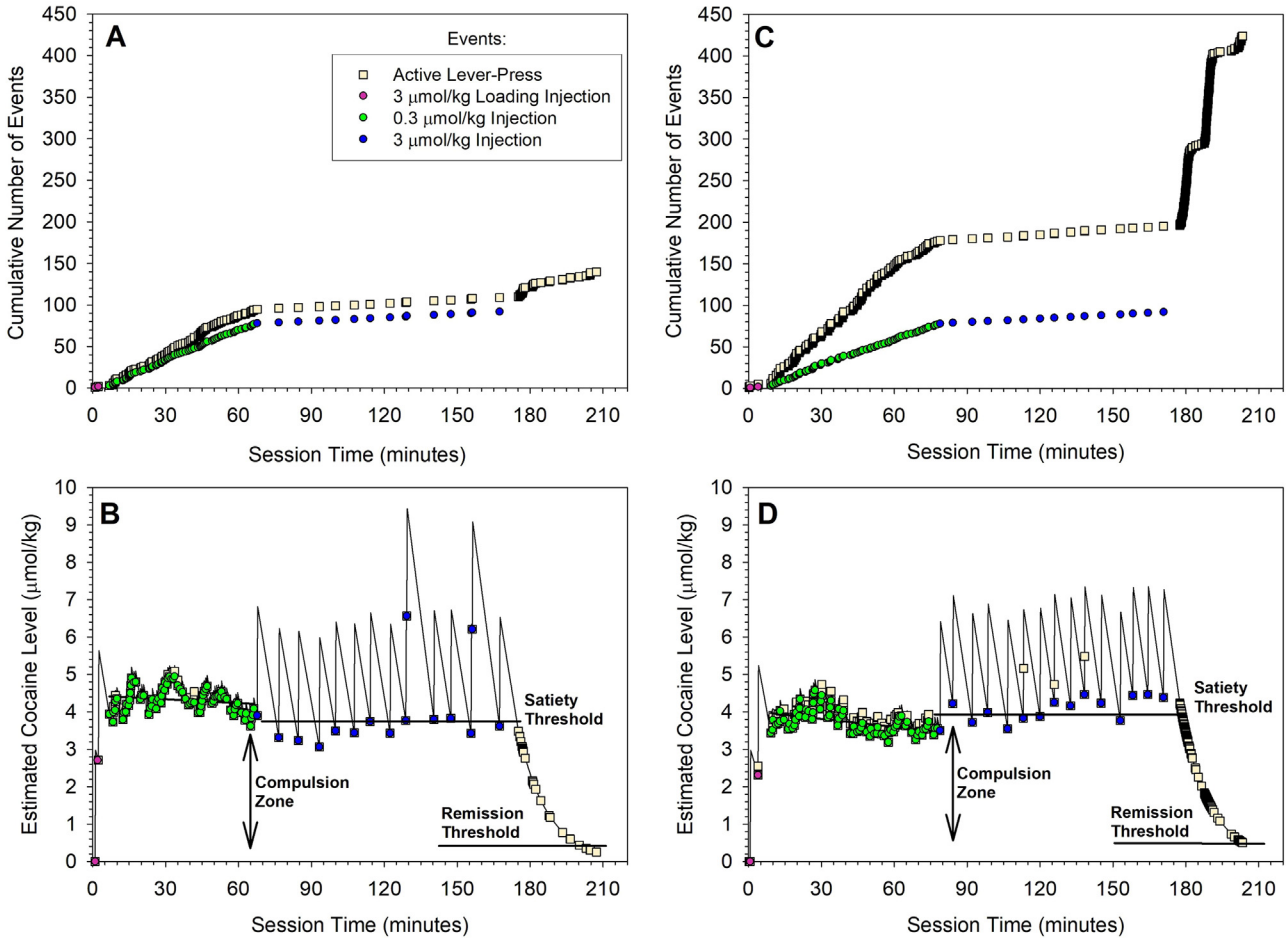

Figure 1. Cumulative record of events (A, C), and estimated level of cocaine at the time of each event (B, D) in representative fixed ratio (FR) 1 sessions from the same rat before (baseline) (A, B) and during exposure to the FR50 schedule (C, D). There were 2 types of events: active lever-presses and drug injections, and A and C plot these events separately. After stably acquiring self-administration behavior, panel A represents a FR1 baseline session using 2 initial self-administered loading doses (3 µmol/kg), then rats self-administered 2 unit doses (0.3 [75 injections] then 3 µmol/kg [15 injections]). After access to cocaine was terminated (unloading), active lever-presses had no consequence but were recorded until lever-pressing stopped. The rat was then switched to FR1/FR50 sessions. During this time, once a week, rats self-administered cocaine on the same FR1 schedule and a representative session is shown in panel C. Panels B and D show the estimated level of cocaine in the body at every second of the same sessions shown in A and C, respectively, and the displayed events are also the same.

ule of cocaine presentation as the FR1 baseline sessions every Monday. These sessions are termed "FR1 during exposure to FR50 schedule." There were 3 FR1 during exposure to FR50 schedule sessions for each of the 8 rats.

After rats maintained responding under high FR schedules of cocaine presentation, responding by the rats was maintained exclusively under the same FR1 schedule of cocaine presentation as the FR1 baseline sessions. These sessions were performed twice a week for up to 7 weeks after exposure to the FR50 schedule ended. During these 7 weeks 3 of the 8 rats lost catheter patency.

FR1/FR50 Sessions

After FR1 baseline sessions, all 8 rats maintained responding under FR schedules of cocaine presentation greater than FR1, starting with FR 5, 10, 20, and lastly 50. These self-administration sessions consisted of either 2 (3 µmol/kg unit dose) or 4 (1.5 µmol/kg unit dose) self-administered cocaine loading injections, followed by 15 injections (3 µmol/kg unit dose) at FR1 and then 15 injections of the same unit dose at the FR schedule greater than FR1. Then access to cocaine was terminated and unloading lever-presses were recorded but had no consequences. There were 1 to 3 sessions for the FR1/5, 10 or 20 sessions and 4 to 6 FR1/FR50 sessions for each of the 8 rats.

Statistical Methods

The parameters measured were interinjection intervals at the 2 unit doses during the FR1 baseline sessions and FR1 during exposure to FR50 sessions, interinjection intervals at the 1 unit dose during FR1/FR50 sessions, and interpress intervals during the unloading phase of all sessions. The estimated cocaine levels at the time of each injection and lever-press was collected from all sessions.

Interinjection intervals, number of lever-presses, and estimated cocaine levels were compared between the FR1 baseline sessions and the weekly FR1 during exposure to FR50 sessions. Interinjection intervals at FR1 and FR50 from the FR1/FR50 sessions were compared. The estimated cocaine levels at the time of injections were also compared in these sessions in addition to the levels at the first of the 50 lever-presses of the FR50 schedule with the FR1. Additionally, the total number of unloading lever-presses, duration of unloading, and the estimated cocaine level at the time of the first and last unloading lever-press were compared between all types of sessions.

All data were analyzed using mixed-effects ANOVA models, with random effects included to reflect the experimental design (ie, different FR schedules or the type of schedule/training preceding the unloading phases were within-animal factors, and there

Table 1
Effect of the unit dose of cocaine on the interinjection intervals and estimated cocaine level at the time of injection during fixed ratio (FR) 1 cocaine self-administration sessions before (baseline) and during exposure to FR50.*

	Unit dose, μmol/kg	FR1 baseline	FR1 during exposure to FR50 schedule
Interinjection interval, min	0.3	0.9 (0.04)	0.8 (0.03)†
	3	6.0 (0.2)	6.4 (0.2)
Total No. of lever-presses during the phase	0.3	279 (8)	331 (14)
	3	40 (3)	46 (3)
Cocaine Level at injection, μmol/kg	0.3	3.9 (0.02)	4.4 (0.03)‡
	3	4.6 (0.06)	4.4 (0.06)

* Values represent the mean (SEM) from 3 sessions from each of 8 rats. These data are from sessions conducted as shown in the representative sessions in Figure 1.
† All values from FR1 sessions during exposure to FR50 schedule were compared with the corresponding FR1 baseline sessions; there were no significant differences.
‡ Not significantly different from cocaine level at 3 μmol/kg per injection for FR1 baseline sessions, and FR1 sessions during exposure to FR50 schedule.

were multiple sessions per animal). Residual variance terms were specified as heterogeneous across sessions to ensure that each session was weighted in inverse proportion to the observed variation within the session, which we expected to vary substantially among sessions. The resulting estimates of mean response under different conditions are reported along with 95% CIs, and differences among conditions were regarded as significant if the corresponding test statistic had $P < 0.05$, 2-sided.

Results

Maintained cocaine self-administration on FR1 schedule before and during exposure to the FR50 schedule

Interinjection intervals were regular at 0.3 μmol/kg unit dose in FR1 baseline sessions (Figure 1A) with a mean of 0.9 minutes (Table 1). When the unit dose was increased to 3 μmol/kg, interinjection interval remained regular (Figure 1A) but the mean increased to 6.0 minutes (Table 1). The regularity in interinjection interval (Figure 1C) and mean values at each unit doses were not altered in the FR1 sessions while rats were exposed to training on the FR50 schedule (Table 1).

Lever-pressing on FR1 schedule before and during exposure to the FR50 schedule

In the FR1 baseline sessions during access to the 0.3 μmol/kg unit dose, the rate of lever-pressing was slightly higher than the rate of injections. These additional presses occurred during the 5 second timeout period. At the higher unit dose, there were less additional lever-presses compared with the lower unit dose (Figure 1A and Table 1). In contrast to the lack of change in the interinjection intervals of self-administration, the number of lever-presses during exposure to FR50 schedule increased, and this effect was especially noticeable at the lower unit dose (Figure 1C and Table 1). Again, these additional lever-presses occurred during the timeout period.

Cocaine level at the time of injection during maintained cocaine self-administration on FR1 schedule before and during exposure to the FR50 schedule

Despite the 6.7-fold higher rate of self-administration at the 0.3 μmol/kg compared with the 3 μmol/kg unit doses in FR1 baseline session (Figure 1A and Table 1), the estimated cocaine levels at the time of injection were similar across these unit doses (Figure 1B and Table 1) and averaged around 4 μmol/kg. Similarly, the lack of dose dependency on estimated cocaine level at the time of injection was also similar in FR1 sessions during exposure to FR50 schedule (Figure 1D and Table 1), and the mean cocaine levels were not altered when rats were exposed to the FR50 schedule (Table 1). Lever-presses during timeout occurred within a narrow range of cocaine levels close to the level at the time of each injection.

Table 2
Interinjection interval, estimated cocaine level at injection (fixed ratio [FR] 1 and FR50), and at the first of the 50 lever-presses at FR50 during FR1/FR50 cocaine self-administration sessions. The cocaine unit dose was 3 μmol/kg.*

	FR1	FR50
Interinjection Interval, min	5.93 (5.58–6.28)	7.70 (7.35–8.05)†
Cocaine level at injection, μmol/kg	4.86 (4.81–4.91)	3.52 (3.49–3.55)‡
Cocaine level at the first of the 50 lever-presses, μmol/kg	N/A	3.81 (3.75–3.87)§

N/A = not applicable.
* The values represent the geometric mean (lower–upper limits of the 95% CI) from 4 to 6 sessions from each of the 8 rats. These data are from sessions conducted as shown in the representative session in Figure 2.
† Significantly different from the corresponding FR1 value, $P < 0.0001$.
‡ Significantly different from the corresponding FR1 value, $P < 0.0001$.
§ Significantly different from the cocaine level at injection at FR1, $P < 0.0001$.

Effect of FR50 on interinjection intervals compared with FR1

All interinjection intervals during the FR1/FR50 sessions were regular (Figure 2A). The interinjection intervals on FR1 at 3 μmol/kg were similar in FR1/FR50 sessions (Table 2) and FR1 sessions before and during exposure to the FR50 schedule (Table 1). When the schedule was switched from FR1 to FR50 in FR1/FR50 sessions, the interinjection interval remained regular (Figure 2A) but increased by 30% from a mean of 5.9 to 7.7 minutes (Table 2).

There were few additional lever-presses at FR1. However, as required, there were at least 50 lever-presses between each injection at FR50, in addition to any timeout presses. The rate of lever-pressing for the 50 required lever-presses was consistent (Figure 2B), and the duration of these 50 presses averaged approximately 1.8 minutes.

Effect of FR50 on cocaine level at the time of injection

When the schedule was changed to FR50, each injection occurred at a lower concentration compared with that at FR1 (Figure 2C). Obviously, the cocaine level at the first lever-press following an injection at FR50 was higher than that at the time of injection, and was closer to the level at the time of injection at the FR1 part of the session. The mean level at first lever-press follow-

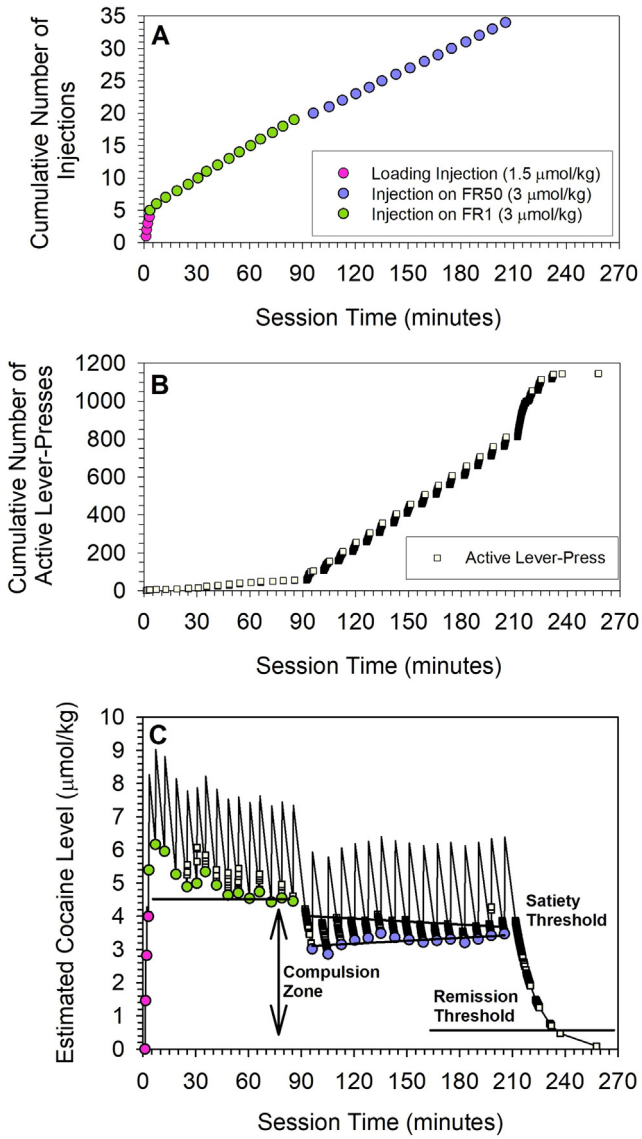

Figure 2. Cumulative record of injections (A), active lever-presses (B), and the estimated level of cocaine at the time of each event (C) during a representative FR1/FR50 session. After fixed ratio (FR) 1 baseline sessions, rats were switched to sessions in which the unit dose 3 µmol/kg was self-administered on the FR1 schedule then the schedule was changed to FR5, 10, 20, or 50. This figure shows a representative FR1/FR50 session. After 4 self-administered loading injections of 1.5 µmol/kg, 15 unit doses were self-administered for each schedule. After access to cocaine was terminated (unloading), active lever-presses were recorded until lever-pressing stopped but had no consequence.

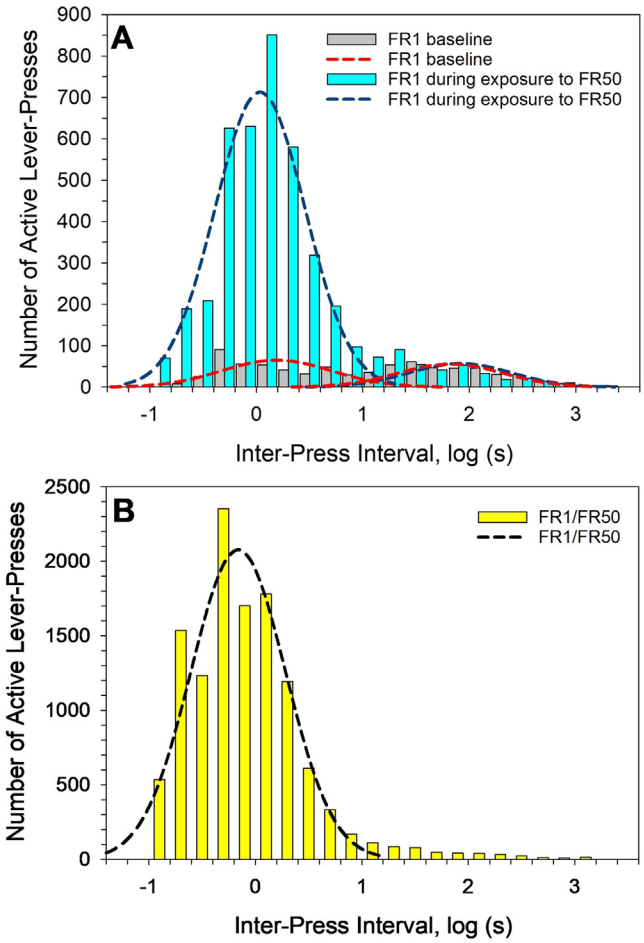

Figure 3. Distribution of interpress intervals (IPIs) for fixed ratio (FR)1 baseline sessions and during exposure to the FR50 schedule (A), and for FR1/FR50 sessions (B) after access to cocaine was terminated and lever-presses had no consequence (unloading). The IPIs are from 3 sessions per rat, from each of the same 8 rats for the FR1 baseline and during exposure to FR50 schedule sessions. The IPIs for FR1/FR50 sessions are from 4 to 6 sessions per rat from the same 8 rats. Because the number of sessions were different from FR1 baseline and during exposure to FR50 schedule, they are shown separately in panel B. Distributions were lognormal and bimodal. However, in panel B, 92% of IPIs could be accommodated in the unimodal model shown.

ing injection at FR50 was significantly lower than the mean level at the FR1 injections, but only by 21% (Table 2).

The mean estimated cocaine level at the time of injection was 28% lower at FR50 (3.5 µmol/kg) relative to FR1 (4.9 µmol/kg). However, the concentration of cocaine at the time of the first lever-press in the sequence of 50 (3.8 µmol/kg) was closer to the concentration at the time of injection at FR1 (Table 3).

Lever-pressing behavior when access to cocaine was terminated

The highest rate of lever-pressing behavior during a session was observed when access to cocaine was terminated (Figures 1A, 1B, and 2B). Once lever-pressing commenced, an average of 23 lever-presses occurred during an average of 29 minutes before rats stopped lever-pressing (Table 3). After exposure to the FR50 schedule, the number of lever-presses observed after access to cocaine was terminated dramatically increased (Figure 1B). The mean number of lever-presses increased from 23 to 158 (Table 3). Despite the approximately 6-fold increase in number, the duration of lever-pressing was unchanged (Figure 1B and Table 3). Similarly, after access to cocaine was terminated in the FR1/FR50 sessions, the number of lever-presses was even higher (Figure 2A), and was a mean of 245 (Table 3). Despite the 9.7-fold increase in the number of lever-presses relative to FR1 baseline sessions, the duration of lever-pressing was also unaltered (Table 3).

The level of cocaine at the time of the first lever-press after access to cocaine was terminated was similar across all sessions (Table 3) and was comparable to the level at the time of injections during cocaine self-administration at FR1. Lever-pressing behavior continued as cocaine levels declined. The cocaine level when lever-pressing stopped was also similar across all sessions irrespective of the schedule (Table 3).

The range of interpress interval after access to cocaine was terminated in FR1 sessions before exposure to the FR50 schedule was broad—from 0.1 seconds to 1000 seconds (Figure 3A)—and the distributions of interpress intervals was lognormal and bimodal

Table 3
Lever-pressing behavior after access to cocaine was terminated (unloading) in fixed ratio (FR) 1 sessions before (baseline) and during exposure to FR50 schedule and FR1/FR50 sessions.*

	FR1 baseline	FR1 during exposure to FR50 schedule	FR1/FR50
No. of unloading lever-presses	23 (13–40)	158 (90–278)†	245 (142–423)†
Duration of unloading lever-pressing behavior, min	29 (18–45)[b]	28 (19, 42)[b]	38 (24–60)[b]
Cocaine level at first unloading lever-press, μmol/kg	4.6 (4.1–5.1)[c]	4.4 (3.9–4.9)[c]	4.5 (4.1–4.9)[c]
Cocaine level at last unloading lever-press, μmol/kg	0.6 (0.3–0.9)[d]	0.3 (0.07–0.6)[d]	0.5 (0.3–0.7)[d]

* Values represent the geometric mean (lower–upper limits of the 95% CI) from 3 sessions per rat for FR1 baseline and during exposure to FR50 schedule, and from 4 to 6 sessions per rat for FR1/FR50 sessions from the same 8 rats. Values with different superscript letters (b, c, d) are not significantly different from corresponding values.
† Significantly different from corresponding FR1 baseline value, $P < 0.0001$.

Table 4
The results of the 2-component normal models applied to the log transformed inter-press interval data from Figure 3.*

	FR1 baseline	FR1 during exposure to FR50 schedule	FR1/FR50
Mean 1, sec	0.7 (1.2)	1.0 (1.4)	0.6 (1.5)
Mean 2, sec	28.9 (3.8)	18.6 (4.0)	11.2 (5.1)
Proportion of IPIs in Distribution 1	0.30	0.85	0.92

FR = fixed ratio.
* Values for Mean 1 represents the mean interpress interval (corresponding variance) of the distribution with the shorter interpress intervals. Mean 2 represents the mean interpress interval (corresponding variance) of the distribution with the longer interpress intervals. The proportion values represent the proportion of interpress intervals (IPIs) that fall within the distribution with the shorter interpress intervals.

(Figures 3A and B). Following exposure to the FR50 schedule, the increase in number of presses was accompanied by a dramatic increase in the proportion of shorter interpress intervals (Table 4). The mean of the short interpress intervals was similar in all sessions irrespective of the schedule (Table 4). The mean of the longer interpress intervals tended to shorten after exposure to the FR50 schedule (Table 4).

Schedule-induced increase in lever-pressing behavior was long-lasting

As shown in Figure 4, the elevated number of lever-presses during the unloading phase (after access to cocaine was terminated) remained elevated for at least 7 weeks after the last exposure to FR50 sessions.

Number of lever-presses after access to cocaine was terminated (unloading) depends on the FR magnitude

There was no increase in the number of unloading lever-presses observed in FR1/FR5 sessions relative to FR1-exclusive sessions. However, after FR1/FR10 and FR1/FR20, significant increase in number of unloading lever-presses was observed. There was no apparent difference in the number of unloading lever-presses after FR1/FR20 and FR1/FR50 sessions. The sigmoidal model used here predicts that the number of unloading lever-presses approached the maximum between FR20 and FR50 (Figure 5).

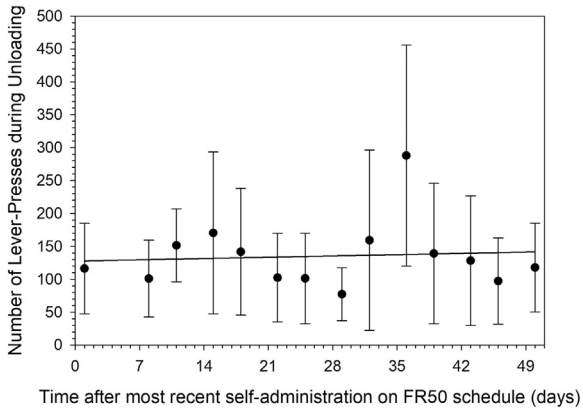

Figure 4. The duration of increase in unloading lever-pressing. After completion of daily fixed ratio (FR) 1/FR50 sessions rats were returned to FR1 only sessions twice per week for up to 7 weeks. Rats displayed high lever-pressing behavior during the unloading phase of these FR1 sessions. Symbols represent the mean (SEM) number of active lever-presses during unloading when access to cocaine was terminated and lever-presses had no consequence (n = 5 rats). The line through the data is a linear regression and shows no evidence of decrease over this time, slope = 0.2803; $P = 0.7804$. For reference, the arithmetic mean (SEM) FR1 baseline value was 32 (6), (n=8 rats).

Discussion

Cocaine-induced lever-pressing behavior in the compulsion zone is increased after high FR schedules

A striking feature of exposing rats to the FR50 schedule is the dramatic increase in the number of lever-presses during the unloading phase (when access to cocaine is terminated and cocaine levels decrease). Regardless of whether rats were on FR1 sessions (Figures 1C and D and Table 3) or on FR1/FR50 sessions (Figures 2A and B and Table 3), there was an approximate 6- to 10-fold increase in the number of lever-presses during the unloading phase. Despite the dramatic increase in the number of cocaine-induced lever-presses, the duration of lever-pressing remained the same. That the duration of lever-pressing during unloading did not change implies that the range of the compulsion zone did not change after exposure to FR50. Consistent with this conclusion,

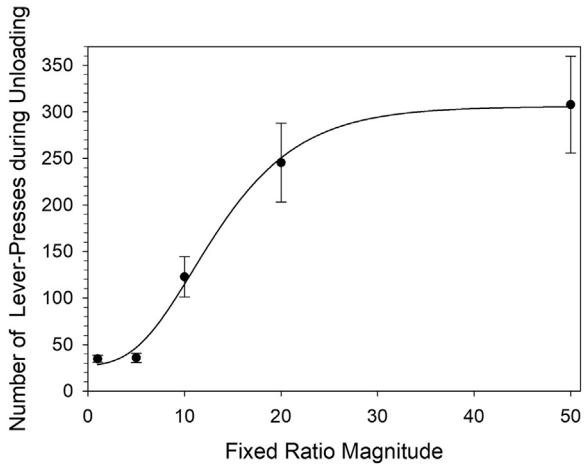

Figure 5. Effect of fixed ratio (FR) magnitude on the number of lever-presses during unloading when access to cocaine was terminated in FR1 baseline, FR1/FR5, FR1/FR10, FR1/FR20, and FR1/FR50 sessions. Symbols represent the mean (SEM) from 4 to 6 sessions (8 rats). The curve represents a best fit sigmoid function, $R^2 = 0.996$.

the estimated cocaine levels at the onset and cessation of lever-pressing, corresponding to the satiety and remission thresholds respectively, remained similar (Table 3). A similar increase in the number of lever-presses and a lack of change in the range of the compulsion zone was also observed after rats self-administered cocaine on a PR schedule or returned to FR1 sessions after exposure to a PR schedule.[14,16]

The dramatic increase in lever-pressing activity during the unloading phase (when access to cocaine was terminated) after exposure to the FR50 schedule was the result of a decrease in the proportion of long interpress intervals and an increase in the number of short interpress intervals. This occurred in all sessions after exposure to high FR schedules. This increase in the number of short interpress intervals was also observed after exposure to PR schedules for cocaine.[16] To our knowledge, this is the first study to document the dramatic increase in lever-pressing behavior after exposure to high FR schedules. We speculate that this phenomenon is previously unreported because investigators rarely return their attention to low FR schedules during their studies,[17] and/or infrequently include as part of their studies the lever-presses after access to cocaine is terminated.[18] Interinjection intervals (rate of cocaine self-administration) were unaltered when responding was maintained under an FR1 schedule of cocaine presentation despite the dramatic increase in the rate of unloading lever-presses after exposure to FR50. This suggests that the increase in cocaine-induced lever-pressing behavior was induced by exposure to high FR schedules and all other relevant effects of cocaine were unaltered. The relevance of this dramatic schedule-induced increase in lever-pressing behavior to the cocaine self-administration paradigm in rats is unclear, although it does improve the resolution of the compulsion zone threshold levels and measurement of the duration of the unloading phase.

Increase in lever-pressing activity had minimal effect on satiety threshold on the FR1 schedule

In addition to the dramatic increase in number of lever-presses during the unloading phase, the number of lever-presses during the maintenance phase at both unit doses also increased in FR1 sessions after exposure to the FR50 schedule. These extra lever-presses were more prominent at the lower unit dose because the number of lever-presses between injections were approximately similar but there were many more injections per unit time at the lower unit dose. Exposure to the FR50 schedule did not result in any change in interinjection intervals at either unit dose on FR1 schedule. Therefore, the additional lever-presses occurred during the timeout period where lever-presses had no consequence. Furthermore, the relative consistency in the level of cocaine at the time of cocaine self-administration demonstrates that the increase in lever-pressing had little, if any, effect on the cocaine satiety threshold. Clearly, it is important to differentiate lever-presses from cocaine injections during maintained cocaine self-administration, especially at lower cocaine unit doses, which are commonly used.[9]

The schedule-induced increase in lever-presses during maintained cocaine self-administration on FR1, makes a small timeout period useful. This allows cocaine to distribute to the brain sufficient to induce a satiety response before the additional lever-presses result in injections. Multiple injections with short intervals followed by long pauses would obscure the effect of unit dose on interinjection intervals and on the level of the satiety threshold.

The increased interinjection interval at FR50

The regularity of the interinjection intervals indicates that the satiety threshold model also applies to the FR50 schedule. This study is the first to investigate the cocaine satiety threshold model at FR schedules greater than FR1. Although regular, interinjection intervals were longer at FR50 compared with FR1. This can be accounted for simply by the time taken for the rats to perform the additional 49 presses, which represents an experimenter-imposed requirement. The duration of the additional lever-presses also accounts for the lower estimated cocaine levels at the time of injection on FR50 compared with FR1. The mean estimated cocaine level at the first of the 50 lever-presses was closer to the level at the time of injection at FR1 (satiety threshold), implying that the first lever-press also represents the satiety threshold. However, the cocaine level at the first of the 50 lever-presses was lower than the level at injection on FR1, so the possibility that the satiety threshold value decreased during FR50 cannot be excluded. If the satiety threshold did decrease, the magnitude was small, and the lack of change in duration of lever-pressing behavior during unloading also indicates that a change in satiety threshold magnitude was negligible, if any.

The schedule-induced effect of increased lever-pressing behavior was long-lasting

Once the high rate of lever-pressing during the unloading phase was established after exposure to the FR50 schedule, it persisted for up to at least 7 weeks in FR1-exclusive sessions (Figure 4). Long-term incubation of craving has also been reported after withdrawal from cocaine self-administration.[19] This phenomenon was related to cue-induced rather than the cocaine-induced lever-pressing in the present study. Unlike a PR schedule, where the rate of lever-pressing during unloading gradually decreased toward baseline levels,[16] there was no evidence of a trend of lever-pressing rate returning to baseline in the present study. Thus, it is possible that the high FR-induced increase in lever-pressing behavior could be irreversible.

Increased rate of lever-pressing during the unloading phase is more prominent after higher FR magnitude

Rate of unloading lever-pressing behavior gradually increased at FR10 and then FR20, and appeared to be maximal between FR20 and FR50. This suggests that increasing FR requirement increased the rate of lever-pressing in the compulsion zone, and the increased rate of lever-pressing increased the probability of the rats maintaining regular cocaine self-administration at the high FR

schedule. It is possible that the number of responses during unloading is maximum immediately after FR50 sessions because this number (SEM), 308 (52), is comparable with observations during a PR schedule, where the mean (SEM) break point at 6 to 12 µmol/kg cocaine unit dose was 364 (71).[14] Additionally, any FR schedule greater than FR20 may have a maximal effect on increasing the rate of lever-presses, whereas FR5 may have little effect on the rate. Indeed, studies using low FR schedules and even FR10[20] did not report this dramatic increase in the rate during unloading. Experimental designs should consider the differences in schedule-induced rates of lever-pressing at different FR magnitudes, especially when the rate of lever-pressing is the dependent variable.

Limitations of the study

The study only used male rats and did not explore if cocaine self-administration in female rats would produce the same results. Another limitation of this study is that rats self-administered cocaine on FR1 and FR50 schedule at only the 3 µmol/kg unit dose. However, the increase in interinjection interval on FR50 vs FR1 was also observed across a range of cocaine unit doses.[21] Additionally, this study explored high FR schedules only up to FR50. Using a higher FR magnitude for cocaine self-administration may determine whether the maximum FR magnitude where cocaine self-administration is maintained by rats is equivalent to the PR breakpoint. Furthermore, rather than measuring plasma or brain cocaine concentrations (technically challenging) we estimated whole body cocaine levels. At the pseudo-steady state during maintained cocaine self-administration at regular intervals, the estimated concentrations in the brain will be different from actual levels by the apparent volume of distribution. However, this will be constant after the maintenance phase of the session is established.

Conclusions

The only consequence of high FR schedules was the increased rate of lever-pressing activity. This was due to a dramatic increase in short interpress intervals with no change or a decrease in long interpress intervals. The relevance of this phenomenon to understanding the mechanisms underlying cocaine self-administration in rats is unclear. Furthermore, the increase in interinjection intervals observed at FR50 is a result of the time to complete the required presses, which is an experimenter-imposed requirement. Because all lever-presses continue to be restricted to the compulsion zone, and the satiety and remission thresholds appear to be largely unchanged, these thresholds appear to be fundamental physiological constants in rats that have acquired cocaine self-administration behavior. The compulsion zone theory provides a rational scientific basis for understanding cocaine self-administration behavior.

Declaration of Competing Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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