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Examination of behavioral strategies regulating cocaine intake in rats

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Abstract

Rationale—It has long been observed that rats self-administer psychostimulants in a highly regular pattern. The inverse relationship between dose and rate of drug intake has been interpreted as a titration phenomenon wherein brain-cocaine levels are maintained within a range. Most studies examining this phenomenon have used fixed, unit doses in which case the only titration strategy available to the animal is to adjust inter-infusion intervals.

Objectives—In this study we examined whether selection of dose size could also be a factor in regulation of intake. We used a schedule of reinforcement, under which the dose can vary through a wide range and is determined by the behavior of the animal.

Methods—Rats self-administered cocaine using a behaviorally dependent dosing schedule of reinforcement, under which the size of each dose was determined by the length of time the lever was held down. The concentration of cocaine was changed across sessions.

Results—Total pump-time self-administered decreased by 56% following each doubling of the concentration, which led to an average 11% increase in total intake. Similarly, estimated brain levels of cocaine increased by 12% for each doubling of concentration. These adjustments were the result of manipulation of both the size and spacing of infusions.

Conclusions—In agreement with previous studies, the regular pattern of intake appears to be the result of a titration mechanism in which animals maintain brain levels of cocaine above some threshold. Compensatory regulation appeared to involve both the selection of dose size and inter-infusion intervals.

Keywords

Cocaine; self-administration; regulation; estimated brain levels; modeling; dose; inter-infusion interval; rat

Introduction

The pattern of cocaine self-administration has long been studied to understand the mechanisms controlling drug intake. It has been recognized that the rate animals self-administer psychostimulants such as cocaine and amphetamine is inversely related to the size of the unit dose (Gerber and Wise 1989; Pettit and Justice 1991; Pickens and Thompson

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1968; Wilson et al. 1971) which is the preselected dose per injection usually held constant throughout the session. The fact that the total amount of drug per session is relatively stable regardless of the size of the unit dose has been offered as evidence that drug intake is somehow titrated. Further experimental evidence has added great weight behind this titration hypothesis by showing that amphetamine levels (Yokel and Pickens. 1974), estimated cocaine levels (Tsibulsky and Norman 1999), and nucleus accumbens dopamine levels (Ranaldi et al. 1999; Wise et al. 1995) – typically thought to play a critical role in the reinforcing effects of psychostimulants – are maintained above a theoretical threshold during self-administration sessions. The titration hypothesis is consistent with behavioral data showing that animals typically self-administer several unit doses quickly at the beginning of the session, which has been called the “loading” phase. A maintenance phase follows during which injections are regularly spaced (Ettenberg et al. 1982; Pickens and Thompson. 1968; Wilson et al. 1971; Wise et al. 1995).

Previous studies have typically held the unit dose constant (but see for example Gerber and Wise 1989; Pettit and Justice 1991); however the development of a behaviorally dependent dosing (BDD) schedule provides a method to address whether dose is a factor in the regulation of intake. When the unit dose is held constant, it is not available as a dependent variable. Thus the only titration strategy available to the animal is to adjust the inter-infusion intervals and spacing of injections. A BDD schedule described by Morgan et al. (2009) allows the behavior of the animal to determine the injection size. On this schedule, the duration that the syringe-pump is activated is contingent upon a hold down response rather than a simple lever press. That is, the syringe pump is directly controlled by depression of the lever, such that the duration of each infusion is determined by the length of time the lever is held down. Morgan et al. (2009) demonstrated that manipulation of the concentration of cocaine in the syringe led to proportional changes in responding; and, similar to findings from fixed unit dose studies, the total intake per session remained relatively constant (Morgan et al. 2009). It is important to note that both dose size and inter-infusion interval can be used as dependent variables on the BDD schedule. This provides a unique opportunity of examining the regulation of drug intake in a situation where both variables can vary and interact with each other.

We recently used a BDD procedure to examine the relationship between estimated brain levels (EBLs) of cocaine and the size of the self-administered dose (Zimmer et al. 2011). EBLs were calculated using equations adapted from standard pharmacokinetic principles (Pan et al. 1991) that have been used in many behavioral self-administration studies (Ahmed and Koob 2005; Samaha et al. 2002; Zernig et al. 2007; Zimmer et al. 2011, 2012). The accuracy of these equations have been demonstrated using electrophysiological (Nicola and Deadwyler 2000; Peoples and Cavanaugh 2003; Peoples et al. 2007; Peoples et al. 2004), voltammetric (Hermans et al. 2008; Stuber et al. 2005a; Stuber et al. 2005b), and microdialysis (Wise et al. 1995) techniques. Forced timeout periods were introduced into the session to allow EBLs to decline to predictable levels. It was shown that the self-administered dose at the beginning of each trial was highly correlated with EBLs. That is, larger doses – sometimes as large as 4 mg/kg - were self-administered when EBLs were very low. It appears that the size of the loading dose closely matched the amount necessary to elevate drug levels into a theoretically preferred range. This single large dose phenomenon parallels the loading phase observation when animals group multiple fixed unit doses together (Ettenberg et al. 1982; Pickens and Thompson, 1968; Wilson et al. 1971; Wise et al. 1995).

The present study extended these findings to an analysis of the maintenance phase. If animals can indeed titrate their intake on a BDD procedure we would expect to see a relationship between the size of each selected dose and subsequent inter-infusion interval.

On a BDD procedure dose size is adjusted by either holding the lever down for a long duration or by emitting a large number of very small responses in a short period of time. We have observed previously that rats self-administering cocaine appear to use the latter strategy, responding in clusters rather than a single, long response (Zimmer et al, 2011). In this study we undertook a rigorous analysis of these infusion-clusters to determine the most appropriate criterion to classify which infusions constitute a cluster. Animals were given unrestrained access on a BDD schedule with various concentrations of cocaine, and the subsequent infusion clusters and inter-cluster intervals (ICIs) were measured. Results show that both clusters and ICIs changed in response to concentration manipulations. Additionally, the magnitude of the cluster self-administered was found to correlate with the size of the subsequent (but not previous) ICI. These results offer support to the hypothesis that EBLs of cocaine are maintained within an elevated zone, and show that both dose and ICI are modified in response to concentration changes.

Methods

Animals, surgery, and housing

Male, Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing approximately 350 g were used for all experiments. Following a seven day acclimation period, a chronic, indwelling Silastic cannula was implanted into the right jugular vein. The catheter exited through the back of the animal in the region of the scapulae. Animals were anesthetized during surgery by a combination of ketamine (100 mg/kg; *i.p.*) and xylazine (8 mg/kg; *i.p.*), and ketoprofen (5 mg/kg; *s.c.*) was used as a post-operative analgesic. After surgery, animals were housed in a stainless steel operant chamber (30 × 30 × 30 cm), and were allowed to recover from surgery for at least 3 days. Room lights were on a 12 hour reverse light/dark cycle (lights on at 3 PM), and food and water were provided *ad libitum*. All procedures were approved by the Wake Forest University Animal Care and Use Committee and followed all NIH guidelines outlined in the Guide for the Care and Use of Laboratory Animals.

Self-administration training

During the training period, a single press on the operant response lever (fixed ratio - FR1) resulted in a 0.75 mg/kg infusion of cocaine delivered over ~4 seconds depending on the weight of the animal. A 20 second timeout period followed each injection, during which the lever was retracted and a stimulus light above the lever was illuminated. Six-hour training sessions began six hours into the dark cycle and took place seven days/week. Training was complete when the animal self-administered the maximum (20) number of infusions on two consecutive days and displayed a stable pattern of responding. After training acquisition, all animals were trained to self-administer cocaine on a BDD schedule of reinforcement (5 mg/ml concentration). All animals continued on this schedule until responding was stable, which was defined as self-administering ~ 1.0 ml or more per day (~40 sec of pump-time) for three consecutive days.

Behaviorally Dependent Dosing (BDD) schedule of reinforcement

Under the BDD schedule of reinforcement, the syringe-pump was activated for the duration that the lever was depressed. Releasing the lever caused the syringe-pump to immediately switch off. Therefore the magnitude of the infusion was directly related to the length of time that the lever was held down. The concentration of cocaine was 10, 5, 2.5, or 1.25 mg/ml depending on the experimental condition, and the pump speed was held constant at 1.6 ml/min. The stimulus light was only illuminated when the lever was in a down state.

Estimated brain level (EBL) calculation

A two-compartment model was used to calculate whole-brain levels of cocaine as previously

described (Pan et al. 1991). The equation $C = \frac{dk}{v(\alpha - \beta)}(e^{-\beta t} - e^{-\alpha t})$ was used where d represents the dose self-administered, k is the rate constant representing the flow between the two compartments [0.233 min^{-1}], v represents the apparent volume of the brain [0.15 L Kg^{-1}], α and β [0.642 and 0.097 min^{-1} respectively] are constants that represent the redistribution and elimination of cocaine respectively, and t (minutes) is the time since the previous infusion. The time course of brain levels for each infusion was calculated independently with a five second resolution and then summed.

Drug

Cocaine hydrochloride (National Institute on Drug Abuse Research, Research Triangle Park, NC, USA) was dissolved in physiological saline and passed through a microfilter ($0.45 \mu\text{m}$). All drug concentrations are represented as the weight of the salt.

Statistics and data analyses

The primary dependent measures were total session intake (mg), estimated brain levels (EBLs – as calculated above), inter-cluster intervals and infusion-clusters. Infusion-clusters were defined as the total mg of a sequence of infusions that occurred within a short period of time as previously described. This is a necessary calculation when using the BDD schedule of reinforcement as it has been shown that animals self-administer large clusters of infusions rather than holding the lever down for a single period of time (Zimmer et al. 2011). The criteria used to determine which infusions should be grouped together were varied systematically in this study (see Figures 2 and 4). A repeated measure one-way ANOVA was used on datasets that passed the Shapiro-Wilk test for normality. A Friedman repeated measures ANOVA on ranks was performed on datasets that failed the normality test. Post-hoc evaluations were made using the Holm-Sidak or Neuman-Keuls method. Correlation

coefficients and their corresponding t values ($t = r \sqrt{\frac{n-2}{1-r^2}}$) were determined using Microsoft Excel (2007), and all ANOVA tests were performed using SigmaPlot (version 11). The probability (p) level was set to 0.05.

Results

All animals ($n = 10$) were offered each concentration (1.25, 2.5, 5 and 10 mg/ml) of cocaine for three days under a BDD schedule of reinforcement. Figure 1a shows cumulative records for an example animal. Decreasing the cocaine concentration in the syringe produced a relatively proportional increase in responding illustrated in Figure 1A by a steeper slope of the cumulative record. This apparent compensation is quantified in figure 1B which shows the mean total pump-time for all rats across each concentration. Data failed the Shapiro-Wilk normality test. A Friedman repeated measures ANOVA on ranks revealed a significant difference between concentrations (chi-square(3) = 28.92, $p < 0.001$), and a Newman-Keuls post-hoc analysis demonstrated a significant difference between all comparisons. This response pattern led to a relative equalization of intake within the session. Figure 1C shows the mean total intake per session for all animals at each of the four concentrations. Data failed the Shapiro-Wilk normality test. A Friedman repeated measures ANOVA on ranks revealed a significant difference between concentrations (chi-square(3) = 16.44, $p < 0.001$), and a Newman-Keuls post-hoc analysis demonstrated a significant difference between every concentration (all six pair-wise comparisons).

Figure 2 illustrates the clustered pattern of responding on a BDD schedule of reinforcement. The top line shows the self-administration pattern of an animal within an 80 second period during the middle of the session. Forty responses occurred during this period. In our previous study (Zimmer et al. 2011), a relatively arbitrary criterion of 20 seconds was chosen to define a cluster. That is, any responses occurring within 20 seconds of each other were combined. In the present study, an analysis of several criteria ranging from 1–30 sec was performed in order to characterize the most appropriate cluster criterion. Combining events that occurred within one second of each other produced four clusters and the intervals in between these clusters are shown (Fig 2A - top). Note that increasing the criterion to 20 or 30 would reduce the number of clusters to 2 and 1 respectively. The bottom line in Figure 2A shows a higher resolution of the responses that occurred during a 4 second period. Within this period the pump was activated 12 times for a total duration of 2.25 sec. Figure 2B shows the average number of clusters calculated for all rats at all concentrations using a 0, 1, 10, 20 or 30 sec cluster criterion. Note that applying a 1 sec criterion combines many responses together and increasing the cluster criteria beyond 1 sec has less dramatic effects.

Figure 3 shows the EBLs of cocaine during representative sessions of one rat self-administering cocaine at each of the 4 concentrations (Fig 3, A–D). Horizontal lines represent the mean EBL for the session. Note that at all concentrations, EBLs fluctuated around the mean with relatively little variability, and there was little difference between mean EBLs despite an 8-fold change in concentration. Figure 3F shows the relationship between infusion-clusters (1 sec cluster criteria – see methods) and EBLs. The same data represented in Fig3D are plotted at a higher resolution (minutes 60–90) and the corresponding infusion-clusters that occurred during that period are on the lower panel of 3F. The average maintained EBLs for all rats at all concentrations are shown in figure 3E. Data failed the Shapiro-Wilk normality test. A Friedman repeated measures ANOVA on ranks revealed a significant difference between concentrations ($\chi^2(3) = 15.60$, $p < 0.001$), and a Newman-Keuls post-hoc analysis demonstrated a significant difference between 5/6 comparisons (no statistically significant difference was observed between 2.5 vs 5.0 mg/ml).

Figure 4 shows the relationship between cluster size and subsequent inter-cluster interval (ICI) for an example session using all five cluster criteria (Fig 4, A–E). Note the low correlation value observed between the two variables when no cluster criterion was applied (panel A). A stronger relationship between cluster size and ICI emerged when any of the five cluster criteria were used (panels b–e). Each animal ($n = 10$) self-administered cocaine at each of the four tested concentrations for three days, yielding a total of 120 self-administration sessions ($10 \text{ rats} \times 4 \text{ concentrations} \times 3 \text{ days}$). Sessions were excluded if daily intake was below 0.2 ml. A correlation coefficient was determined for each day, and the statistical significance of each point was determined by calculating the t-statistic and corresponding probability (p , one-tailed). The results from our correlation analysis are displayed in figure 4F. Cluster criteria had relatively little effect on the number of sessions that showed a significant relationship (88% were significant with no cluster and 84–96% were significant using a criteria of 1–30 sec). However, applying a cluster criterion had an effect on the magnitude of the average r value as shown in figure 4F. Non-significant sessions were often due to an outlying ICI (*i.e.* a 20 min period of non-responding).

Analysis of selected infusion-clusters compared to the previous ICI revealed a weaker relationship. The average magnitude of the significant correlations was much lower than those calculated for infusion-cluster vs subsequent ICI. Specifically, with a cluster criterion of 0, 1, 10, 20 or 30 sec the mean r value for cluster vs previous ICI was 0.20, 0.29, 0.29, 0.31 and 0.31 respectively. For cluster vs subsequent ICI the mean was 0.30, 0.57, 0.56, 0.58 and 0.55 respectively.

Because titration of EBLs across concentrations could be accomplished by either adjusting the size of the selected infusion-cluster or the length of the ICI, an analysis of both of these variables was performed. The mean ($\pm$ SEM) pump duration and corresponding dose at each concentration with either a 0 or 1 sec cluster criterion is shown in Fig 5A and 5B respectively. When clusters were not accounted for, a one-way repeated measures ANOVA revealed no statistically significant difference in the average pump durations across concentration (Fig 5A, closed circles. $F [9, 27] = 0.13$), which explains why the average dose increased significantly and proportionally to the change in concentration (Fig 5B, closed circles. $F [9, 27] = 72.49$, $p < 0.001$). A one-way repeated measures ANOVA on the average pump duration with a 1-sec cluster criterion revealed a significant difference between concentrations (Fig 5A, open circles. $F [9, 27] = 14.02$, $p < 0.001$). A post-hoc analysis (Holm-Sidak) revealed all comparisons were significantly different except 2.5 vs 5.0 mg/ml, indicating that the average pump duration decreased as concentration of cocaine increased. The corresponding average dose was also significantly different across concentrations, as revealed by a one-way repeated measures ANOVA (Fig 5B, open circles. $F [9, 27] = 33.36$, $p < 0.001$). Post-hoc analysis (Holm-Sidak) revealed a significant difference for all comparisons except 1.25 vs 2.5 mg/ml. The mean ICI also increased as a function of concentration, such that the largest concentrations led to the largest ICIs (Fig 5C). A Friedman repeated measures ANOVA on ranks (data failed normality test) revealed a statistically significant difference between concentrations with a 1-sec cluster (chi-square(3) = 22.68, $p < 0.001$) and without clustering (chi-square(3) = 28.92, $p < 0.001$). A Student-Newman-Keuls post-hoc analysis without the clustering criteria demonstrated a significant difference between all comparisons and with a 1-sec cluster all comparisons were significant except 10.0 vs 5.0 and 2.5 vs 1.25 mg/ml.

Discussion

The present study examined the regulation of cocaine self-administration using a novel BDD schedule of reinforcement. This schedule allows for a broad range of doses that depend on the behavior of the animal. One of the unique attributes of this schedule is that the size of dose and inter-infusion interval can both be used as dependent variables. Similar to other schedules of reinforcement (Gerber and Wise 1989; Ranaldi et al. 1999; Tsibulsky and Norman 1999; Wilson et al. 1971; Wise et al. 1995), relatively constant estimated brain levels were maintained within each session on the BDD schedule (Fig 3). Following manipulation of cocaine concentration, compensatory adjustments were seen in both infusion-cluster size and inter-cluster interval (Fig 5B and 5C). Finally, a significant relationship between the size of each cluster and the subsequent (but not previous) ICI was found (Fig 4).

Several mechanisms have been proposed to explain the regulation of psychostimulant intake. One possibility is that cocaine has a direct effect on the motor behavior of animals such that they become incapable of responding for some period of time (Pickens and Thompson 1968). This explanation is unlikely given the results from experiments examining concurrent intracranial self-stimulation (ICSS) and *i.v.* drug self-administration. Wise et al. (1977) reported an augmentation of ICSS rates during post-infusion pauses, demonstrating that rats are fully capable of responding during this interval. An alternate hypothesis is that cocaine levels are maintained within a range - either above a threshold and/or below a ceiling (Tsibulsky and Norman 1999; Wise et al. 1995; Wilson et al. 1971; Pickens and Thompson 1968). It has been demonstrated that cocaine has anxiogenic properties and can produce avoidance behavior (DeVries and Pert 1998; Ettenberg and Geist 1991, 1993) and conditioned taste aversions (Booth et al. 1977; Goudie et al. 1978). However, microdialysis studies of psychostimulant self-administration do not appear to support the idea that dopamine levels fluctuate below a theoretical ceiling (Wise et al. 1995; Ranaldi et al. 1999).

These authors argued that if titration was due to the animal avoiding upper aversive effects, it would be expected that a frequent low dose would be preferred over larger ones. However, animals consistently choose larger doses (Johanson and Schuster 1975; Llewellyn et al. 1976; Lynch et al. 1998; Ward et al. 2005), suggesting that the aversive properties of cocaine cannot fully account for the titration of brain levels. Additionally, a microdialysis study demonstrated that amphetamine levels at the time of each response were relatively constant despite a wide range of available doses suggesting that rats titrate drug levels above a threshold value (Yokel and Pickens, 1974).

The titration hypothesis - that rats self-administer to maintain brain levels of drug above a theoretical threshold - makes several predictions. First, when current drug levels fall below the threshold, the subsequent cluster selected should correlate with the length of the *previous* ICI. That is, the further below the threshold drug levels fall the greater the dose required to return levels to the threshold. Second, when animals are maintaining drug levels above the threshold it would be expected that the size of the dose selected would correlate with the *subsequent* ICI. That is, a large dose would push brain levels significantly above the threshold and animals would wait the appropriate length of time for brain levels to return to the satiety threshold before resuming responding. Our previous report demonstrated that large loading clusters could be induced by introducing forced timeout periods and that the size of these clusters correlated with the length of the previous ICI as predicted (Zimmer et al, 2011). The results of the current study lend support to the interpretation that animals titrate brain levels above a threshold by showing that cluster size correlated more strongly with the subsequent ICI than the previous one when animals were maintaining drug intake with no forced timeouts.

Panlilio et al. (2003) have argued that the titration hypothesis predicts an autocorrelation between sequential latencies. That is, an error in one inter-infusion interval should produce a compensatory adjustment to the next one. However, they were unable to demonstrate such a relationship and concluded that the temporal regularity of responding was not due to compensatory adjustments of inter-infusion intervals. Their failure to observe a significant correlation may be due to the insufficient variability in Inter-infusion intervals produced by using a fixed unit dose. We would argue that the correlations observed in the present experiment using the BDD schedule are due to the much larger variance in dose and ICIs.

There remains one issue that is hard to reconcile with the titration hypothesis. Studies consistently report that EBLs and nucleus accumbens dopamine levels are not constant across a range of doses (Pettit and Justice. 1991; Pickens and Thompson 1968). The present study replicated these observations by showing an increase in mean maintained EBLs and intake with larger concentrations (Figs 1c and 3e). These findings appear to be in contrast to the titration hypothesis, which posits that changes in dose are corrected by a proportional change in responding. One possible explanation of this phenomenon is that the elimination kinetics of cocaine produces a non-linear function of dose and subsequent inter-infusion intervals (Tisbulsky and Norman 1999). This explanation works well with the behavioral data, but is hard to reconcile with dose-dependent nucleus accumbens dopamine levels and estimated brain-cocaine concentrations. Other possible explanations include differences in work output with changing doses or the sensitivity of interoceptive cues.

To the extent that animal studies can provide an understanding of human drug taking, previous research using fixed, unit doses would suggest that spacing of inter-injection intervals is the primary regulatory strategy. The BDD schedule offers a different dimension (manipulation of dose) and points to the fact that animals will self-administer quite different doses throughout the session and use a variety of strategies to regulate their intake. This work would predict that human regulation of cocaine levels would be accomplished by a

combination of adjustments in both the size and spacing of injections. This prediction appears to match human regulation of other drugs such as nicotine. The study of nicotine consumption has confirmed that dose (puff size) and inter-puff interval interact in the regulation of smoking behavior and that smokers appear to titrate nicotine levels (Ashton et al. 1979; McMorro and Foxx 1983; DeGrandpre et al. 1992). To our knowledge clinical studies of cocaine intake have not yet reached this detailed level of analysis.

The present study confirmed previous results that the selected dose on the BDD schedule can be described by clusters of multiple short responses. The first study characterizing the BDD schedule of reinforcement showed animals selecting surprisingly small doses of cocaine (Morgan et al. 2009). Further analysis suggested that multiple responses are typically clustered within a very short period of time (Zimmer et al. 2011). The present study confirmed these findings by showing that the average pump duration for a single response does not change across concentration but the number of these responses occurring within a cluster does (Fig 5a). Further evidence that a single hold down response is not a predictive unit of measure but needs to be grouped together comes from the observation that single responses do not show expected relationships while clusters of responses do. For example, clustering responses that occurred within even 1 sec of each other resulted in a dramatic increase in the predictive ability of clusters on the subsequent ICI (Fig 4). Analyzing the data with multiple cluster criteria demonstrated similar significant results for a range of criteria (1–30 sec). A 1 sec criterion was used for all subsequent analyses as it represented the most conservative option and it appeared that using a larger criterion provided no added advantage and could potentially group unrelated clusters together.

Finally, the use of specific terms in this study deserves comment. A number of proponents of the titration hypothesis have offered theoretical explanations using terms such as “hedonic set point”, “satiety threshold”, “trigger point”, “compulsion zone” etc. (Ahmed and Koob 1998; Wise et al. 1995; Tsibulsky and Norman 1999). These terms have been helpful in focusing attention on core concepts of the titration hypothesis. However, in some cases these terms imply a level of conscious control. We wish to emphasize that the present support for the titration hypothesis should not, by itself, be interpreted as evidence for a specific conscious or emotional state. Homeostatic mechanisms do not necessarily require conscious awareness (e.g. blood pressure), and it remains unclear the degree to which awareness contributes to the regulation of cocaine concentrations in the brain. For example, levels of nicotine appear to be titrated within a narrow range by human smokers without them having an explicit awareness of the phenomenon (Ashton et al. 1979; Gritz et al. 1976; Herning et al. 1985). Some stimuli that control drug seeking and taking are conscious while others are not (Childress et al. 2008). Therefore, it would be inappropriate to presume the processes described here involve conscious awareness, volition or any particular emotional state of the animal. Such conclusions would require additional data.

The present study manipulated cocaine concentrations in order to examine the self-administration behavior on a BDD schedule of reinforcement during the maintenance phase. In agreement with previous studies, animals appear to titrate EBLs around a relatively constant level by adjusting both cluster size and ICI. In addition, a positive correlation between cluster size and the subsequent ICIs was found, which suggests that animals maintain EBLs above some presumed threshold.

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Fig. 1. Self-administration behavior on the behaviorally dependent dosing schedule with four cocaine concentrations. (A) Cumulative responses for a representative animal at each of the concentrations during three hour sessions. (B) Average pump time for all animals at each concentration. (C) Average intake for all animals at each concentration. Statistically different ($p = 0.05$) values are indicated by * different than 1.25 mg/ml, # different than 2.5 mg/ml and † different than 5.0 mg/ml

Fig. 2.

Visual representation of the clustered pattern of responses on the BDD schedule. (A) An event record of an 80 second period within a three hour session for a representative animal responding for cocaine is shown on the top line. The forty responses the animal made in this period were clustered into four bouts. An expansion of the four seconds during which one of these bouts occurred is shown on the bottom line. Within this period the animal activated the pump 12 times for a total 2.25 sec. (B) The average number of clusters for all rats at all concentrations using a 0, 1, 10, 20 or 30 sec cluster criterion.

Fig. 3. Estimation of brain levels of cocaine during BDD self-administration sessions with four different concentrations of cocaine. Brain levels within a session are shown for a representative animal at each concentration (A–D). Horizontal lines represent the average brain level maintained during each session. (E) The average of these means ($\pm$ SEM) for all animals is shown at each concentration. The data from panel D are shown at higher resolution (F) with the corresponding infusion-clusters plotted in the lower panel for comparison. Statistically different ($p = 0.05$) values are indicated by * different than 1.25 mg/ml, # different than 2.5 mg/ml and † different than 5.0 mg/ml

Fig. 4. The correlation between selected cluster size and subsequent inter-infusion interval. (A–E) The relationship between cluster size and subsequent inter-infusion interval for the same example session using all 5 cluster criteria. (F) The average correlation coefficient for all rats at each dose and using each cluster criteria

Fig. 5.

Analysis of dose size and inter-infusion interval. (A) The mean pump duration for all animals at each cocaine concentration without clustering responses (closed circles) or with a 1 sec cluster criterion (open circles). (B) The mean cluster self-administered at each concentration with no cluster criteria (closed circles) or a 1 sec cluster criteria (open circles). (C) The mean inter-cluster interval at each concentration with no cluster criterion (closed circles) or a 1 sec cluster criterion (open circles). Statistically different ($p = 0.05$) values are indicated by * different than 1.25 mg/ml, # different than 2.5 mg/ml and † different than 5.0 mg/ml