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The compulsion zone: A pharmacological theory of acquired cocaine self-administration

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Abstract

In rats trained to reliably self-administer cocaine, the cumulative drug level was calculated during sessions in which cocaine was administered either contingently or non-contingently. During both types of sessions a high rate of responding was observed only when cocaine levels were above the priming threshold but below the satiety threshold. When the levels of non-contingently administered cocaine were maintained between the priming and satiety thresholds for at least 5 h rats continuously maintained high rates of responding. Although it is generally assumed that rats are responding for cocaine during self-administration sessions, the persistence of responding during non-contingent administration is consistent with responding being induced by cocaine. Therefore, in contrast to the basic assumptions underlying the operant theory of self-administration behavior, choice, contingency and reinforcement are not necessary to explain acquired cocaine self-administration. The presented data demonstrate that there is no ascending limb of the dose-response curve and that the cocaine priming and satiety thresholds delineate the lower and upper limits, respectively, of a cocaine "compulsion zone". It is concluded that the self-administration paradigm is the sum of cocaine induced responding and cocaine induced satiety and which of these cocaine-induced effects occur at any time is dependent on the cocaine level. This novel pharmacokinetic/pharmacodynamic theory provides a basis for a comprehensive understanding of the cocaine self-administration paradigm.

Keywords

Pharmacokinetic; Pharmacodynamic; Addiction; Drug abuse; Operant behavior

1. Introduction

Cocaine reliably reinstates (primes) extinguished self-administration in rats trained to self-administer cocaine (De Witt and Stewart, 1981) and this response occurs when the cocaine concentration (level) exceeds the cocaine priming threshold (Norman et al., 1999, 2002). The priming threshold is defined as the minimum concentration of cocaine in the body that will reinstate self-administration (Norman et al., 1999). During the maintained self-administration of cocaine, the regularity and unit dose dependency of self-administration are long established

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(Pickens and Thompson, 1968). Several studies have demonstrated that pharmacological parameters can explain the regularity of maintained drug self-administration. The time between injections of amphetamine could be explained by first order drug elimination and a minimum maintained drug level (Yokel and Pickens, 1974) above which animals are in a state of satiety (Wise, 1987). Therefore, this minimum maintained drug level was termed the satiety threshold and was defined as the maximal level of cocaine at which the probability of self-administration is high and above which the probability of self-administration is low (Tsibulsky and Nor-man, 1999). Furthermore, in that same study, the relationship of the satiety threshold to the drug elimination half-life, the inter-injection interval and the unit dose was defined mathematically. This latter model of maintained self-administration is consistent with the report that plasma concentration of cocaine remained relatively constant during self-administration (Lau and Sun, 2002) and the hypothesis that falling dopamine levels when they reach a certain trigger point induce successive responses in the intravenous cocaine self-administration paradigm (Wise et al., 1995).

Based on the above stated definitions of the priming threshold and the satiety threshold, it can be hypothesized that self-administration occurs only when cocaine levels are between these thresholds. This is consistent with a previous proposal that once brain dopamine levels are slightly elevated by a priming injection or other manipulation, the probability of responding increases to almost one and remains high until levels of drug and nucleus accumbens levels of dopamine are sufficiently elevated (Wise, 1999). Furthermore, when the levels of cocaine at the time of each lever press during self-administration sessions were calculated the result was consistent with this hypothesis (Tsibulsky and Norman, 2005). Therefore, the priming and satiety threshold models may be unified into a comprehensive model of the self-administration paradigm.

It is commonly believed that in the self-administration paradigm the investigator controls the unit dose of drug and the animal controls the time of injection making the inter-injection interval the fundamental dependent measure. According to our unified priming and satiety thresholds model, the level of drug, rather than the unit dose, represents a critical parameter in the self-administration paradigm, therefore, the investigator should control this parameter. This is best achieved by administering the drug non-contingently. The contingency of responding on drug delivery is a central tenet of the operant theory of behavior, of which self-administration is assumed to be an example. However, if the level of drug controls responding, then it is not necessary to assume that the contingency of drug delivery is vital for the maintenance of responding. Therefore, in this study we determined whether rats would maintain lever-pressing behavior if cocaine was administered non-contingently at rates maintaining different minimal levels of cocaine in the body.

For clarification, it should be emphasized that the acquisition of self-administration behavior and environmental cue-induced priming are not included in the scope of this study. However, it is concluded herein that in rats that have acquired stable cocaine self-administration behavior cocaine induces two distinct pharmacodynamic responses relevant to the self-administration paradigm: selective facilitation of lever-presses and selective inhibition of lever-presses. Evidence is presented that the concentration of cocaine determines which of these cocaine-induced responses are observed at any time.

2. Results

2.1. The distinct phases of a cocaine self-administration session

Fig. 1A shows the cumulative event record from a representative session illustrating that several different phases can be distinguished on the basis of the effect of unit dose and contingency on the rate of responding within a cocaine self-administration session. Lever presses induced

by environmental cues extinguished quickly after placing the animals into the experimental chambers. The highest rate of self-administration was observed immediately after reinstatement. During the loading phase, when the programmed unit dose was changed from 43 to 100 μg/kg, the mean inter-injection interval remained constant. However, after five presses at the same unit dose of 100 μg/kg, the rate of self-administration abruptly decreased. This delineated the transition to the maintenance phase of the session. The inter-injection intervals during this maintenance phase were regular at any unit dose and were proportional to the unit dose. Thus, when the unit dose was increased from 100 to 1000 μg/kg the mean interval also increased from 35±3 to 292±11 s, respectively. During the extinction phase, after access to cocaine was terminated, the rate of lever pressing initially increased from that observed during the maintenance phase of the session to the rate observed during the loading phase, then gradually decreased until responding was extinguished.

2.2. The level of cocaine at the time of each lever press during self-administration sessions

Fig. 1B shows the calculated levels of cocaine at the time of each cocaine administration or extinction lever press during the same representative session shown in Fig. 1A. The lowest level of cocaine required for reinstatement of extinguished lever-pressing activity represented the cocaine priming threshold which in this session was 150 μg/kg. After priming occurred, the calculated level of cocaine at the time of each self-administration increased rapidly (to around 1900 μg/kg). The levels of cocaine at the beginning of maintenance then stabilized. When the unit dose of cocaine was increased 10-fold the level of cocaine at the time of each self-administration remained stable (Fig. 1B) whereas the mean inter-injection intervals increased approximately 8-fold (Fig. 1A).

After the initial loading phase was complete, the level of cocaine at the time of each self-administration represented the satiety threshold. Linear regression analysis demonstrated that there was no significant change in the magnitude of the satiety threshold, at either unit dose, for the duration of the maintenance phase of the session where the inter-injection intervals were stable at a particular unit dose (Fig. 1A). The mean levels of cocaine at the time of each self-administration during the maintenance phase of the sessions did not depend on the cocaine unit dose (Fig. 1B). Therefore, data were collapsed across doses for each session and then between sessions for each rat. For this group of 11 rats the mean±S.E.M. satiety thresholds calculated at the time of each self-administration during the maintenance phase of individual sessions and irrespective of the unit dose was 1581±63 μg/kg (Table 1).

The highest rate of self-administration was observed during the loading phase of the session immediately after priming occurred (Fig. 1A). Fig. 2 shows the mean rate of responding during this loading phase of the sessions as a function of unit doses. The unit doses varied between sessions because they represented the ultimate dose of the programmed injections at which priming occurred during individual sessions. There was a poor correlation between the rate of responding and the unit dose (r=0.305) and no evidence that the rate of responding increased as a function of unit dose in this range as indicated by the negative slope of the linear regression line (Fig. 2).

2.3. Responding during the non-contingent administration of cocaine

As shown in Fig. 3, lever-pressing behavior was observed only when cocaine levels were above 300 μg/kg but below 1600 μg/kg. For the group of 11 rats, the mean±S.E.M. duration of responding was 146±8 min. During this time the majority of the presses (293±33) were on the right lever, previously associated with cocaine self-administration, with relatively few presses (31±15) on the left, inactive lever. The cocaine levels were above 1600 μg/kg for approximately the next 3 h, during which time no lever presses were observed. After termination of cocaine injections, lever pressing resumed when the calculated level of cocaine declined below 1600

μg/kg. The last press before the criterion for extinction was reached was at a cocaine level of 550 μg/kg. For the group of 11 rats, the mean±S.E.M. duration of responding during extinction was 42±5 min. During this time the majority of the presses (15±3) were on the right lever and relatively few presses (1.8±0.3) were on the left lever.

During the non-contingent sessions with peak cocaine levels set at 3000 μg/kg, the mean ±S.E.M. cocaine levels at the time of the last press when cocaine levels were rising was 1672 ±81 μg/kg (Table 1). This value was not significantly different (paired *t*-test: n=11, T=0.94, P=0.271) from the mean±S.E.M. cocaine levels (1732±42 μg/kg) at the time of the last press of the loading phase or the mean satiety threshold levels (1581±63 μg/kg, Table 1) of the self-administration sessions. After approximately 3 h without lever presses, lever-pressing behavior was reinitiated when non-contingent injections were terminated and the mean±S.E.M. cocaine levels decreased to 1271±99 μg/kg. Although this value was significantly lower (paired *t*-test: n=11, T=2.6, P=0.016) than the mean satiety threshold, most of the difference was due to two of the rats that reinstated lever pressing at a mean± S.E.M. cocaine level of 813±116 μg/kg (n=2).

2.4. Responding during the non-contingent administration of cocaine that maintained levels below the satiety threshold

As shown in Fig. 4, lever-pressing behavior with intervals shorter than 2 min (a criterion for priming) was observed only when cocaine levels were between 230 μg/kg and preset maximal level of 1000 μg/kg. For the group of 11 rats, the mean±S.E.M. duration of responding was 347±11 min. During this time the majority of the presses (425±73) were on the right lever, previously associated with cocaine self-administration, with relatively few presses (38±20) on the left, inactive lever. After termination of cocaine injections, the last press before the criterion for extinction was reached (30 min with no presses) occurred at a cocaine level of 293±43 μg/kg. This mean value was not significantly different from the corresponding level during extinction of self-administration behavior (321±28 μg/kg, paired *t*-test: n=11, T=0.735, P=0.48).

There was no difference between sessions in terms of the method up to the point when the criterion for the reinstatement of self-administration was met and after the access to cocaine was terminated. The mean values for the first lever press between the self-administration, non-contingent 3000, and non-contingent 1000 sessions were not significantly different and collapsing the data across all sessions gave a mean cocaine level at the first lever press of 185 ±24 μg/kg. The first press did not meet the criterion for the priming threshold in approximately 30% of the sessions. This mean value was significantly lower (paired *t*-test: n=11, T=5.2, P=0.0004) than the cocaine levels when the criterion for priming threshold was met (374±57 μg/kg) reflecting the potential to underestimate the priming threshold during the self-administration sessions because of the increased rate of cocaine injections (approximately every 30 s) relative to the programmed injections (every 2 min).

The mean cocaine levels at which the last press that met the criterion for extinction occurred in the self-administration or any of the non-contingent sessions were not significantly different. Collapsing these values across all sessions for this group of 11 rats, the mean±S.E.M. cocaine level at the time of the last lever press was 330±27 μg/kg. This mean value was not significantly different from the geometric mean value for the priming threshold (374±57 μg/kg, paired *t*-test: n=11, T=0.7, P=0.5).

2.5. Comparison of the cocaine priming and satiety thresholds between self-administration and non-contingent sessions

For both the self-administration and non-contingent sessions the distribution of the values for the priming threshold between sessions was log-normal, consistent with earlier findings (Norman et al., 2002). As shown in Table 1, the mean± S.E.M. of the geometric means from the individual animals were 298±26 and 374±57 μg/kg for the self-administration and non-contingent sessions, respectively. These values were not significantly different (paired *t*-test: n=11, T=2.0, P=0.072).

The mean values for the satiety threshold from the individual animals were 1581±63 and 1672 ±81 μg/kg when measured in the self-administration and non-contingent sessions, respectively (Table 1). These mean values were not significantly different (paired *t*-test: n=11, T=1.6, P=0.13).

3. Discussion

3.1. The probability of a response is determined by the cocaine concentration, not by the cocaine unit dose

Consistent with the definitions of the priming threshold and satiety threshold, lever-pressing behavior occurred only when cocaine levels were at or between these thresholds. Therefore, this range of cocaine levels constitutes operationally a “response zone” and when cocaine levels are above this response zone they are in a satiety zone. This suggests that responding may be all-or-nothing depending upon the cocaine concentration in the body. The drug unit dose is considered an important parameter that determines the rate of responding during self-administration. However, according to the response zone model, the unit dose is only important in relation to how it influences the cumulative concentration of drug. When drug levels increase above the response zone into the satiety zone, the intervals between self-administrations are determined by the time required for the levels to decline back to the response zone, which is described mathematically by the satiety threshold model of maintained self-administration (Tsibulsky and Norman, 1999; Norman and Tsibulsky, 2001; Norman et al., 2004). Another central tenet of the operant theory of self-administration is that rates of responding should increase as a function of unit dose. However, the rate of self-administration according to the satiety threshold model is inversely proportional to the unit dose, which is inconsistent with the predictions made by operant theory. This observed inconsistency has long been recognized and has been interpreted as representing the “rate limiting” or “direct effects” of cocaine (Johanson and Fischman, 1989; Mello and Negus, 1996; Lynch and Carroll, 2001). This resulting descending limb of the dose–response curve for self-administration is a problem that must be resolved for the operant theory of self-administration but is naturally explained by the satiety threshold model of maintained self-administration.

The highest rates of responding were observed when cocaine levels remained within the responding zone throughout the time of measurement. Therefore, the direct, rate-limiting, effects of cocaine should be negligible and the rate-increasing effects of cocaine as a function of unit dose should be observed. This is generally reported as the ascending limb of the dose–response curve (Mello and Negus, 1996). However, the lack of positive correlation between the unit dose and the rates of self-administration during the loading phase of the session is not consistent with there being an ascending limb of the dose–response function. Therefore, there is no part of the unit dose–response function during any phase where self-administration occurs that is consistent with the predictions made by the operant theory of the regulation of self-administration behavior.

The initial high rate of responding during the extinction phase, when access to drug is terminated, is also not accounted for by the operant theory of self-administration. Indeed, it was concluded that the response rate during extinction does not appear to be a reliable indicator of reinforcement magnitude (Yokel and Pickens, 1976). The only observation during a self-administration session that appears consistent with operant theory is the eventual cessation of lever pressing, which is assumed to represent learning of the lack of contingency between responding and drug delivery. Alternatively, according to the responding zone model, the response rate is high because cocaine concentrations are between the priming and satiety thresholds and the extinction of responding occurs when cocaine levels fall below the priming threshold. If so, this raises the intriguing question of whether contingency between responding and drug delivery is necessary for the maintenance of responding.

3.2. Responding is independent of the contingency of cocaine delivery

When cocaine was delivered non-contingently in an escalating dose regimen high rates of responding occurred only when cocaine levels were within a specific range of levels. Importantly, the upper limit of this range corresponded to the satiety threshold and the lower limit, not surprisingly, corresponded to the priming threshold observed in the self-administration sessions. Therefore, the magnitude of the “response zone” did not depend on the contingency of the cocaine delivery. Furthermore, this is consistent with the cocaine level, not the unit dose, being the major determinant of the probability of responding.

That no responses were observed for several hours when cocaine levels were above the satiety threshold is consistent with the previous reports using continuous infusions of cocaine at a constant rate that maintained steady state levels above satiety threshold (Tsibulsky and Norman, 1999) in sessions in which rats could self-administer cocaine at any time. Therefore, the satiety effect is also independent of contingency.

As stated earlier, it is generally believed that contingency is a critical determinant of response rate because discontinuation of injections or substitution of saline for drug solution during self-administration sessions produced an initial response rate increase followed by the cessation of responding. Indeed, in both the self-administration and the non-contingent sessions a high rate of responding resumed after the delivery of cocaine was terminated and remained high for approximately 25–45 min. During this time cocaine concentrations declined from the satiety threshold to the priming threshold level. The complete extinction of responding occurred when cocaine level declined below the priming threshold. In this case, this level could be termed the extinction threshold. This suggests that the responses ceased because the cocaine concentration declined to a level below that required to induce this behavior. The lack of extinction of lever pressing behavior for several hours when non-contingent injections maintained cocaine levels within the “response” zone is consistent with the theory that high rates of responding are maintained only when cocaine concentrations are within a specific zone.

It may be argued that the persistence of responding during non-contingent cocaine administration was due to “intermittent reinforcement” because at least some lever presses occurred by chance immediately before programmed injections. It has been demonstrated that responses reinforced intermittently are very resistant to extinction (Humphrey, 1939). However, it should be noted that the intermittent reinforcement paradigm is recognized to be inconsistent with operant theory and is considered to be a paradox. It may also be argued that the difference between the two kinds of sessions was not whether the delivery of cocaine was contingent versus non-contingent but rather whether cocaine was delivered on a “fixed ratio 1” versus “variable ratio 4” reinforcement schedule. However, if the response rate was unaltered when contingency was 100% or 0%, it can be concluded that contingency does not play a significant role.

Other evidence that contingency is important in the maintenance of self-administration behavior is that in a two-lever cage, the subject responded only on the lever producing the injection, but when the lever contingencies were reversed, the lever preference also reversed (Pickens and Thompson, 1968). This was interpreted as consistent with the explanation of drug self-administration as an operant behavior where drugs of abuse serve as reinforcers (Clark et al., 1961; Weeks, 1962; Pickens and Thompson, 1968). Although the present studies do not address this reversal of preference, this can be interpreted as the ability of rats to acquire a new behavior. The role of contingency in the acquisition of a new behavior is part of the Pavlovian classical conditioning paradigm, but this should not be confused with the role of contingency in determining the probability of an already acquired behavior to occur according to Skinnerian operant behavior theory.

3.3. The cocaine compulsion zone theory as a paradigm shift

From a psychological perspective, where it assumed that the animal is motivated to press a lever for cocaine, the range of cocaine concentrations at which responses occur would represent a “drug-seeking” zone. Alternatively, there is an increasing recognition of the importance of pharmacokinetic and pharmacodynamic parameters in the regulation of drug self-administration (Ahmed and Koob, 2005). From a pharmacological perspective, where it is assumed that drugs induce a defined response, the range of concentrations would represent a “cocaine-induced response zone”. The persistence of responding in the absence of contingency is consistent with responding being induced by cocaine. Because cocaine induces lever pressing behavior, choice is irrelevant and the behavior is compelled. If so, rats do not press the lever for cocaine, they press the lever because of cocaine. This represents a departure from the assumptions underlying the operant theory of drug self-administration behavior. It is concluded that the range of cocaine levels with lower and upper limits delineated by the cocaine priming and the cocaine satiety thresholds, respectively, constitute a “compulsion zone”. According to this compulsion zone theory, once the behavior is acquired the self-administration paradigm consists of two distinct cocaine-induced responses: (1) cocaine-induced facilitation of acquired responding and (2) cocaine-induced selective inhibition of the same responding. Which of these distinct responses is observed at any time depends on whether cocaine concentrations are within or above the compulsion zone, respectively.

It can be seen from this compulsion zone theory that the self-administration of cocaine is a special case where behavior results in the administration of cocaine. The cocaine self-administration paradigm can therefore be envisaged as a positive feedback loop between lever-pressing response to cocaine and the administration of cocaine that maintains levels within the compulsion zone thereby inducing lever-pressing behavior. This pharmacokinetic/pharmacodynamic theory of self-administration unifies the priming threshold and the satiety threshold models (Tsibulsky and Norman, 1999; Norman et al., 1999, 2002) and provides a basis for a comprehensive understanding of the cocaine self-administration paradigm.

As operant behavior theory of self-administration is not consistent with our experimental findings the absolute rate of responding may not be an important parameter and its significance should be reevaluated. The compulsion zone theory treats the rate of responding as an all-or-nothing function of the cocaine level and states that cocaine-induced responding, whatever the rate, only occurs when cocaine levels are within the compulsion zone. However, the absolute rate of responding when cocaine levels are within the compulsion zone varies between sessions and between individual animals. The absolute rate of responding may be additionally regulated by factors other than the cocaine level. For example, the rate of responding is higher during the loading phase compared to the extinction phase, which indicates that the direction of change in cocaine levels may influence the response rate. Importantly, there was no evidence that the different rates were dependent on the contingency of drug delivery.

The compulsion zone theory can explain the effect of ratio and interval schedules of reinforcement on the rate of responding. Briefly, the number of responses per session depends on the product of the absolute rate of responding and the total time that cocaine levels are within the compulsion zone. Schedules with ratio greater than 1 and/or time out greater than 0 restrict access to cocaine thereby increasing the time that cocaine levels remain within the compulsion zone. The compulsion zone theory predicts that the progressive ratio break-point should occur when cocaine levels fall below the priming threshold before all required responses are completed. Ultimately, schedules with very high ratios or very long intervals represent extinction schedules.

According to operant theory, extinction represents the time to learn that contingency has been terminated. If so, the time to complete this process should be relatively constant and independent of the drug. In contrast, the compulsion zone theory predicts that the time for responding to extinguish should be proportional to the time that drug levels are within the compulsion zone. Indeed, we have demonstrated previously that the cocaine analog WIN 35,428 has a 6-fold longer elimination half-life than cocaine and requires 6–8 h to extinguish responding compared with 30–50 min for cocaine (Norman et al., 2004).

It should be emphasized that the compulsion zone theory only explains the self-administration paradigm once the behavior is acquired and established. The theory does not explain the acquisition of cocaine self-administration behavior where rats learn to press a particular lever that is associated with drug delivery.

3.4. Summary

The concentration of cocaine is fundamental to the regulation of cocaine self-administration behavior. The data reported herein are consistent with a compulsion zone with lower and upper limits delineated by the cocaine priming and satiety thresholds, respectively. Cocaine-associated lever pressing behavior is induced by cocaine only when cocaine levels are within this zone.

4. Experimental procedures

4.1. Cocaine self-administration training

Male Sprague–Dawley rats (Sasco, Portage, MI, initial weight 180–220 g and 400–500 g over the duration of the studies) were housed individually on a 12-h light–dark cycle (lights on at 6 a.m.) and food and water were available ad lib. All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Rats were surgically implanted with an indwelling catheter into the right jugular vein under halothane anesthesia (Caine et al., 1993). Beginning six or 7 days after the surgery, rats were trained to self-administer cocaine HCl (500 μg/kg) using a fixed ratio (FR=1) schedule with no time out period (TO=0) after the injection of cocaine is completed. It is well established that external cues associated with drug delivery can maintain responding. For example, lights accompanying injections during the acquisition of self-administration can then maintain high levels of responding in the absence of drug delivery under second order schedules. To minimize the influence of such cues, rats in the present studies were trained to self-administer cocaine in the absence of any sound or light signals. In addition, no signals were used during any part of these studies. Furthermore, no priming injections of cocaine were given to facilitate learning. Under these conditions it was reasonable to expect that the training phase of the study might be extended. Therefore, long overnight sessions during the rat's active period were used assuming that this would increase the rate of acquisition. Sessions started around 18:00 and lasted 14 h every night. In addition, daily test sessions were conducted between 10:00 and 12:00 to test acquisition. The nightly sessions were discontinued when rats demonstrated regular lever pressing activity or

when 25 injections of 500 μg/kg were self-administered during the subsequent test session. The criterion for acquisition (cocaine levels were maintained above the projected priming threshold value of 300 μg/kg) was reached after 33.5±3.6 days (mean±S.E.M., n=11 rats) with a range of 12 to 58 days. Training at the standard unit dose continued until individual rats met the criterion for stable maintained self-administration. This criterion was no significant change of the mean and standard deviation (S.D.) of the inter-injection intervals between five consecutive 2-h daytime sessions.

Twelve test chambers (modified chambers from Lafayette Instrument, Lafayette, IN) were each equipped with an active and an inactive lever. Each chamber was situated inside of a laminated wooden compartment (43×61×35 cm) that provided sound attenuation and was equipped with a house light (7 W). Infusion pumps (model PHM-100, Med Associates, Georgia, VT) were situated outside of the laminated compartments and additionally insulated to attenuate pump noise and vibration. Computers controlled unconditioned stimuli (drug infusions) using a program written in Medstate Notation® language (Med Associates, Inc.). The unit dose of cocaine was regulated by the duration of the injection. Each session began with an activation of the pump for 1.16 s, which filled the dead volume of the catheter (13 μl) with cocaine solution (concentration 5 μg/μl in saline, rate of injection 11.6 μl/s). Catheter patency was evaluated by administration of short-acting barbiturate methohexital (Brevital, 6 mg/kg i.v.) as described before (Caine et al., 1993; Norman et al., 2002).

4.2. Calculation of cocaine levels in the body

Cocaine level in the body (L) was calculated every Δt seconds according to the simplified linear equation of the first order elimination/zero order input kinetics for a two-compartment model (Tsibulsky and Norman, 2005):

$$L_{c1}=L_{c0}(1-k_{10}\Delta t-k_{12}\Delta t)+L_{p0}k_{21}+V_{01}\Delta t \quad (1a)$$

$$L_{p1}=L_{p0}(1-k_{21}\Delta t-k_{20}\Delta t)+L_{c0}k_{12} \quad (1b)$$

where L_{c1} and L_{p1} are cocaine levels at the time t_1 in central and peripheral compartments, respectively, L_{c0} and L_{p0} are cocaine level at the time t_0, $\Delta t=t_1-t_0$ is a computation interval, k_{10}, k_{20}, k_{12} and k_{21} are the cocaine elimination rate constants (obviously $k=\ln(2)/t_{1/2}$), and V_{01} is the rate of cocaine injection. Theoretically, the smaller the value of Δt the better is the estimation for current cocaine levels. The deviation of the simplified linear model from the exponential model was set to not exceed 0.3%. The group mean value for $t_{1/2}$ was approximately 480 s, as calculated using Eq. (2) (Tsibulsky and Norman, 1999). The values for distribution and redistribution half-lives were set at 120 s (Booze et al., 1997) and the value for Δt was set at 1 s. The values for the constants were set as k_{10} =0.0014441, k_{12} = k_{21} =0.005776 and k_{20} =0 s^{-1}, and therefore elimination from the second compartment was assumed to be negligible.

4.3. Satiety threshold measured at different doses of cocaine

Trained rats were placed in test chambers with no priming injections or special signals. Contextual cues induced lever pressing. The dead space of the catheters was filled and FR=1, TO=0 schedule started. The first four doses were the standard 500 μg/kg. The program then changed the unit dose and the next 75 doses were 100 μg/kg. This was done to allow animals to load cocaine level quickly to the satiety threshold and to assure the stability of that self-maintained level. Then the unit dose was programmed to change again and rats could self-

administer a defined number of injections. The permitted numbers of injections varied according to the unit dose and were 50, 25, 15, 10 or 7 injections at unit doses of 250, 500, 1000, 2000 or 4000 μg/kg, respectively. After the defined number of injections were self-administered the program terminated access to cocaine. During this extinction phase as well as during the loading and maintenance phases of the session the time of all lever presses and the corresponding calculated level of cocaine were recorded. The session was stopped 30 min after the last lever press. The time from the termination of cocaine access to the last lever press of the session, the extinction phase, typically was 25–45 min.

After 1 week of measurement of intervals at different unit doses when doses were presented in pseudorandom order, the design of the daily sessions was changed. The sessions started with extinction of cue-induced lever presses and then the priming threshold was measured by non-contingent priming injections as described in Section 4.4. As the cocaine level increased towards the expected satiety threshold during the self-administration of the last programmed dose, the unit dose was switched to 100 μg/kg for 75 self-administrations and the unit dose was then changed to one of the unit doses from 250 to 4000 μg/kg as described above. Inter-injection intervals (T) were measured during the maintained self-administration of the unit doses between 250 and 4000 μg/kg and the satiety threshold (D_{ST}) and the elimination half-life ($t_{1/2}$) were calculated using non-linear regression analysis according to the equation:

$$T=\ln(1+D_U/D_{ST})t_{1/2}/\ln 2 \quad (2)$$

where D_U is the unit dose of cocaine (Tsibulsky and Norman, 1999).

4.4. Priming threshold measured by escalating doses of cocaine

At the start of each daily session (started in the morning between 8:00 and 10:00 and conducted 5 days a week), rats were placed in individual experimental cages and the time of each lever press was recorded. Initial lever presses, presumably induced by environmental cues, complicated the measurement of the cocaine-induced priming threshold. This activity was extinguished by programming the lever presses to have no consequence. Thirty minutes after the last lever press (active or inactive), the catheter was filled and non-contingent injections of escalating doses of cocaine were administered every two min until self-administration behavior was reinstated. Each subsequent injection of cocaine was at an escalated dose calculated to raise the peak cocaine level by 20 μg/kg above the previous peak level. This escalating dose regimen resulted in a linear increase in the peak level as a function of time at the rate of 10 μg/kg per min (Norman et al., 2002).

The criterion for reinstatement of cocaine self-administration was defined as maintained lever pressing after the first five consecutive lever presses occurred at intervals not longer than two min. Once lever-pressing behavior was reinstated, the calculated peak cumulative cocaine levels resulting from the ultimate and penultimate priming injections were recorded and the mean of these values represented the priming threshold. The reinstatement phase of the session followed by the loading phase when the cocaine unit dose was the same as the ultimate priming dose. The number of loading doses was calculated to allow the cocaine level to reach the preset level of the average satiety threshold (2000 μg/kg). After the loading was finished, the session continued as during the sessions for dose–response measurements. The rats self-administered cocaine on an FR=1, TO=0 schedule for approximately 120 min at different cocaine unit doses. The infusion pump was then switched off and the session was terminated after lever-pressing behavior extinguished as described in Section 4.3.

4.5. Non-contingent administration of cocaine

After priming and satiety threshold values were determined the non-contingent sessions were initiated and were alternated with the self-administration sessions. The sessions started with extinction of context-induced lever presses and priming injections of escalating doses of cocaine as it was in the sessions for measurement of the cocaine priming threshold. However, when presses on the active lever were reinstated they remained without any consequences and the programmed injections continued every 2 min until the cocaine level peaks reached 3000 μg/kg. After that the cocaine dose of 460 μg/kg remained constant for 1 h keeping the cocaine levels fluctuating between 2530 and 3000 μg/kg. The extinction phase followed the maintenance phase and lasted until 30 min without any lever presses elapsed. During some sessions the peak levels of cocaine were kept at 1000 μg/kg for 4 h.

4.6. Statistics

The data were visually analyzed using SigmaPlot graphic software (SPSS, Chicago, IL). The Kolmogorov–Smirnov goodness of fit test for candidate distributions and the least-squares linear regression analysis was performed using Statistica package (StatSoft, Tulsa, OK). Student *t*-test and paired *t*-test was used to estimate the significance of difference between parameters measured for different groups of animals and for the same animals, respectively. The level of significance was set at $\alpha=0.05$.

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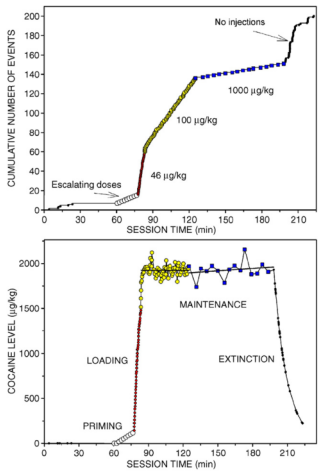

Fig. 1.

Cumulative record of events (A) and the calculated levels of cocaine (B) at the time of each event during a representative self-administration session. Panel A: each vertical increment represents an event and the horizontal distance between each event represents the inter-event interval. Closed circle symbols (●) represent lever presses on the active lever not followed by cocaine injection during the extinction phase. Open circle symbols (○) represent programmed priming injections of escalating doses of cocaine (doses increased from zero to 46 µg/kg). The subsequent self-administration was under a FR 1, no time out schedule. The mean±S.D. inter-injection intervals were 35±3 s and 292±11 s at unit doses of 100 µg/kg and 1000 µg/kg, respectively. The linearity of the data points demonstrates the regularity of the inter-injection intervals during the loading phase (the unit doses were 46 µg/kg, small open circle symbols (○), and 100 µg/kg, larger open circle symbols (○)) and during the maintenance phase (the unit doses were 100 µg/kg, larger open circle symbols (○), and 1000 µg/kg, closed square symbols (■)). Panel B: the calculated cumulative level of cocaine at the time of the initiation of each programmed priming injection and each self-administration of cocaine and also at the time of each lever press during the extinction phase. Linear regression lines demonstrate the stability of cocaine levels at the time of injections during the maintenance phase regardless of the 10-fold difference in the cocaine unit doses (100 and 1000 µg/kg). After the pump was switched off, the level of cocaine declined and all lever presses were recorded until the behavior was extinguished (30 min without any presses).

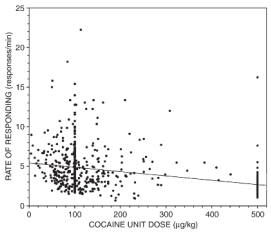

Fig. 2.

The rate of responding during the loading phase of self-administration sessions as a function of the unit dose. The symbols represent the mean inter-response interval during the loading phase of each session. The loading phase was defined by the time from the priming response to the time at which the intervals at a given unit dose abruptly increased. The initial unit dose during the loading phase was equal to the last non-contingent dose at which reinstatement of self-administration occurred, as described in Section 4, and was therefore proportional to the magnitude of the priming threshold. The line represents the best-fit linear regression model, which indicated a significant negative correlation between the two parameters (r=0.305, n=595, P<0.01).

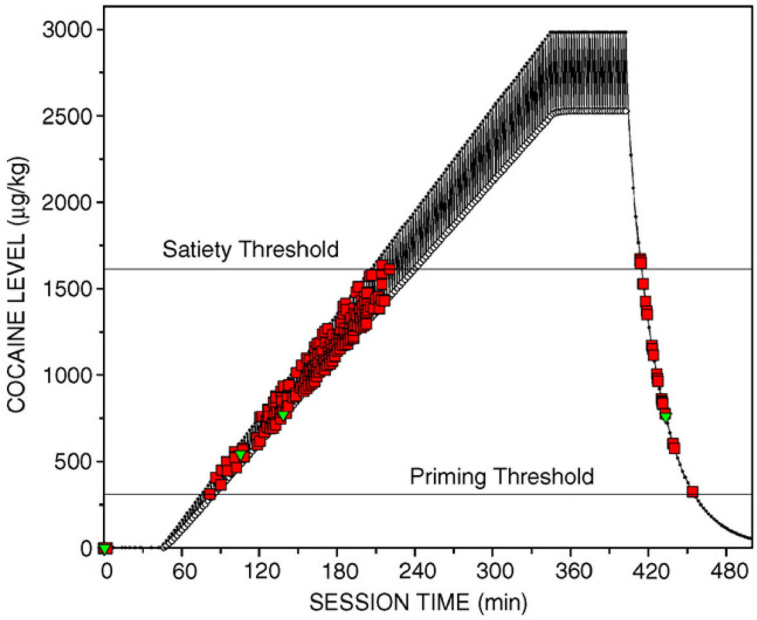

Fig. 3.

Calculated cocaine levels and lever-pressing activity during a representative session with non-contingent injections of cocaine. This representative rat, which previously reliably self-administered cocaine, received programmed cocaine injections every 2 min (represented by the open circle symbols (○)). The line represents the fluctuation of cocaine level between the non-contingent injections and the cocaine level during extinction. Red rectangular symbols (□) represent presses on the previously active lever (*n*=316). Green triangular symbols (△) represent presses on the inactive lever (*n*=4). The program calculated each dose of cocaine to produce an increase in the peak cocaine level at the rate of 10 µg/kg per min. The peak cocaine level was then maintained at 3000 µg/kg for 1 h. During the phase of the session where cocaine levels were increasing, the first and the last lever presses occurred at the cocaine levels of 310 µg/kg (represented by the lower horizontal line—the priming threshold) and 1612 µg/kg (represented by the upper horizontal line—the satiety threshold), respectively. During the phase of the session where cocaine injections were terminated and levels declined, the first and the last lever presses occurred at the cocaine levels of 1674 µg/kg and 325 µg/kg, respectively. [For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article.]

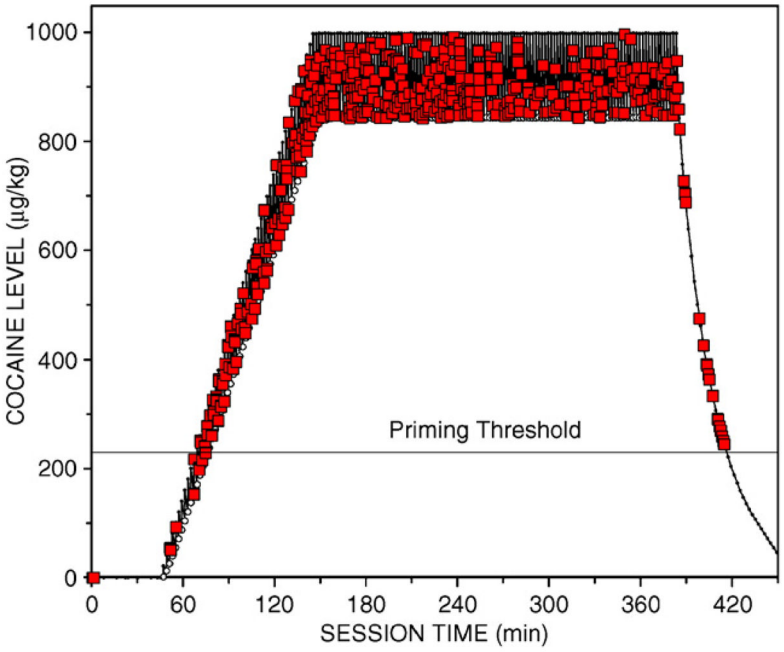

Fig. 4.

The effect on lever pressing activity of maintaining cocaine levels between the priming and satiety thresholds with non-contingent injections of cocaine. Line and symbols are the same as in Fig. 3. The difference is that the peak level of cocaine was maintained at 1000 µg/kg for 4 h. Although the first lever press during the loading phase occurred at a cocaine level of 54 µg/kg the criteria for the priming threshold occurred at a cocaine level of 230 µg/kg (represented by the horizontal line—the priming threshold). The last lever press during the extinction phase occurred at the cocaine level of 245 µg/kg. There was a total of 617 presses on the active lever and no inactive lever presses.

Table 1

Comparison of the priming and satiety threshold estimates between the self-administration sessions and the non-contingent administration sessions measured in individual animals

Rat	Estimates of the priming threshold						Estimates of the satiety threshold					
	Self-administration			Non-contingent			Self-administration			Non-contingent		
	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	S.E.M.	*n*	Mean	S.E.M.	*n*
1	277	248–308	18	288	255–324	17	1376	55	18	1443	88	9
2	189	152–235	11	193	167–220	12	1374	46	10	1652	85	6
3	209	192–228	22	239	219–261	22	1440	43	21	1363	89	13
4	272	230–323	23	175	141–216	9	1824	76	16	1869	137	8
5	275	241–313	14	386	332–450	10	1966	47	14	2203	67	6
6	247	192–317	12	334	267–410	8	1504	125	9	1859	93	7
7	303	246–371	14	253	201–315	17	1815	63	9	1819	109	9
8	466	411–528	23	750	602–877	7	1598	49	23	1823	170	8
9	414	368–467	20	621	550–688	14	1472	64	19	1595	76	10
10	310	246–390	15	306	229–398	11	1665	71	15	1367	92	10
11	321	265–389	13	564	507–627	12	1359	36	12	1404	45	12
Group mean±S.E.M.	298±26			374±57			1581±63			1672±81		

The units of all values are μg/kg of cocaine. The individual mean priming threshold values represent the geometric means with their associated 95% confidence intervals (95% CI) from the indicated number of sessions (*n*) for each animal. The individual mean satiety threshold values represent the mean±S.E.M. from the indicated number of sessions for each animal. The group means represent the mean±S.E.M. of the means from the 11 animals. There was no significant difference in the geometric mean priming threshold values measured in the self-administration and the non-contingent sessions (paired *t*-test: T=2.011, P=0.072). There was no significant difference in the mean satiety threshold values measured in the self-administration and non-contingent sessions (paired *t*-test: T=1.649, P=0.13).