Neuropsychopharmacology (2012) 37, 1901–1910

www.neuropsychopharmacology.org

The Motivation to Self-Administer is Increased After a History of Spiking Brain Levels of Cocaine

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Recent attempts to model the addiction process in rodents have focused on cocaine self-administration procedures that provide extended daily access. Such procedures produce a characteristic loading phase during which blood levels rapidly rise and then are maintained within an elevated range for the duration of the session. The present experiments tested the hypothesis that multiple fast-rising spikes in cocaine levels contribute to the addiction process more robustly than constant, maintained drug levels. Here, we compared the effects of various cocaine self-administration procedures that produced very different patterns of drug intake and drug dynamics on Pmax, a behavioral economic measure of the motivation to self-administer drug. Two groups received intermittent access (IntA) to cocaine during daily 6-h sessions. Access was limited to twelve 5-min trials that alternated with 25-min timeout periods, using either a hold-down procedure or a fixed ratio 1 (FR1). Cocaine levels could not be maintained with this procedure; instead the animals experienced 12 fast-rising spikes in cocaine levels each day. The IntA groups were compared with groups given 6-h FR1 long access and 2-h short access sessions and two other control groups. Here, we report that cocaine self-administration procedures resulting in repeatedly spiking drug levels produce more robust increases in Pmax than procedures resulting in maintained high levels of cocaine. These results suggest that rapid spiking of brain-cocaine levels is sufficient to increase the motivation to self-administer cocaine.
Neuropsychopharmacology (2012) **37,** 1901–1910; doi:10.1038/npp.2012.37; published online 28 March 2012

Keywords: cocaine; self-administration; spiking; loading; maintenance; behavioral economics

INTRODUCTION

Drug addiction is a multifaceted disorder that presents with a variety of symptoms. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), a diagnosis of drug dependence requires the presentation of any three of seven distinct symptoms (American Psychiatric Association, 1994). These range from purely consumptive measures (eg, increased intake) to motivational criteria such as an inability to abstain. The diversity of these criteria suggests that drug dependence involves multiple neurobiological processes that may manifest differently in different individuals (Koob and Volkow, 2010). A comprehensive study of these underlying neurobiological mechanisms requires animal models that reflect the multiple addiction processes.

A number of cocaine self-administration procedures have been developed in rodents to assess specific DSM-IV symptoms associated with cocaine addiction (Vanderschuren and Everitt, 2004). The schedules and access conditions used, and the resulting patterns of cocaine intake, greatly affect the expression of the symptoms. It is important to note that many access conditions produce remarkably stable patterns of drug intake with no apparent change in motivational measures (Roberts *et al*, 2007). This demonstrates that simply allowing an animal to self-administer cocaine is not sufficient to produce an addicted phenotype. Successful rodent models of the progression of cocaine addiction have identified a number of critical features including increased daily access (Ahmed and Koob, 1998, 1999), intermittency (Morgan *et al*, 2002; Morgan and Roberts, 2004), abstinence (Grimm *et al*, 2001; Pickens *et al*, 2011), and speed of drug injection (Liu *et al*, 2005b; Wakabayashi *et al*, 2010).

A long access (LgA) procedure in which rats self-administer cocaine during daily 6-h sessions on a fixed ratio 1 (FR1) schedule is perhaps the most widely used model for examining changes in self-administration behavior over

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Received 28 December 2011; revised 17 February 2012; accepted 27 February 2012

time (Koob and Kreek, 2007; Koob and Volkow, 2010; Zernig *et al*, 2007). This procedure has been shown to produce a robust escalation in drug intake (~30–40%) over a 2-week period, which is not observed in short access (ShA) controls given access to only 1 h/day (Ahmed and Koob, 1998). These sessions, and all FR1 sessions, typically show a loading and maintenance phase (Ettenberg *et al*, 1982; Wilson *et al*, 1971). That is, animals load up at the beginning by self-administering several injections in a short period of time, and for the remainder of the session, infusions become more evenly spaced resulting in cocaine levels being maintained within relatively narrow limits (Ahmed and Koob, 2005). As both LgA and ShA sessions provide animals with the opportunity to engage in loading behavior, the critical difference between these procedures is the length of the maintenance phase. Therefore, it would appear that the escalation effect is due to brain levels of cocaine being maintained for an extended period of time.

Although the LgA model in rodents, in many respects, mimics a 6-h cocaine 'binge' (Ahmed, 2005), clinical data suggest that humans take cocaine in a somewhat different pattern. Recent survey data from cocaine users indicate that experienced individuals consume the same amount of cocaine in a similar period of time as less experienced ones. However, more experienced subjects reported getting significantly fewer 'uses' (<4) from each purchase indicating that, compared with less experienced users, they were self-administering larger doses separated by longer intervals between intoxicating events (Beveridge *et al*, 2012). The inter-use-interval was often much greater than an hour. Given that the half-life of cocaine in humans is ~40 min (Javaid *et al*, 1983), blood concentrations are not maintained at a high level; instead, substantial reductions in blood levels occur which then 'spike' with each intense intoxicating event. It is likely, therefore, that there are qualitative differences in the dynamics of cocaine levels between human drug users and subjects in typical FR cocaine self-administration experiments.

The clinical data suggesting that addicts exhibit spiking rather than relatively stable blood levels during a binge prompted this study. Here, we investigated the effects of 2 weeks of exposure to a cocaine self-administration procedure that engendered a spiking pattern of cocaine intake. Rats were given intermittent access (IntA) to cocaine during 5-min trials that alternated with 25-min timeout periods. This IntA procedure, which was developed while investigating the relationship between the preferred dose of cocaine and the amount of drug on board, results in substantial fluctuations in cocaine brain levels (Zimmer *et al*, 2011). The IntA procedure offers the opportunity to test whether maintained levels are necessary for a change in addiction phenotype and whether rapid spikes in brain levels are sufficient. Two IntA groups were included in the present experiment; during each 5-min trial one group was given access to cocaine using a hold-down (HD) procedure (IntA-HD) while the other group was given access to cocaine on an FR1 schedule (IntA-FR). These two groups were compared with LgA, ShA, and two other control groups. After 2 weeks of daily access to cocaine, the performance of the six groups was compared using a within-session threshold (TH) procedure (Oleson *et al*, 2011). Pmax, defined as the unit-price at which maximal responding occurs (Hursh, 1991), was assessed using a behavioral economic analysis of data. Here, we report that cocaine self-administration procedures resulting in repeatedly spiking drug levels produce more robust increases in Pmax than procedures resulting in maintained high levels of cocaine.

MATERIALS AND METHODS

Animals, Surgery, and Housing

The Wake Forest University Institutional Animal Care and Use Committee approved all experiments before the study commenced. Male Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing approximately 350 g at the time of surgery were used as subjects. Before entering the study, rats were anesthetized with ketamine (100 mg/kg) and xylazine (8 mg/kg) and implanted with chronically indwelling Silastic cannulae (CamCaths, Cambridgshire, UK) as previously described (Liu *et al*, 2007). On recovery, animals were individually housed in $30 \times 30 \times 30$ cm experimental chambers located in a temperature-controlled room (20–21 °C) maintained on a 12-h light–dark cycle (lights on at 1500 hours). A counterbalanced fluid swivel (Instech Laboratories, Plymouth Meeting, PA) mounted above the experimental chamber was used to connect an infusion pump (Razel Scientific Instruments, Stamford, CT) to the cannula using Tygon tubing enclosed within a stainless steel tether. Cannulae were flushed daily with heparinized saline to help maintain patency. Food and water were available *ad libitum*.

Self-Administration Training

After a 3- to 5-day recovery period, all animals were given access (FR1) to a cocaine-paired lever which, when depressed, initiated an intravenous injection of cocaine (0.75 mg/kg, infused over 4 s). When each infusion was initiated, the lever was retracted and a stimulus light above the lever was illuminated, signaling a 20-s timeout period. Sessions occurred 7 days per week and began in the middle of the dark cycle. Sessions were terminated after a maximum of 20 infusions or after 6 h, whichever occurred first. An animal was considered to have acquired if 20 injections were self-administered for 2 consecutive days and a stable pattern of post-infusion pauses was apparent. Following training, separate groups of animals were assigned to one of six daily access procedures (ShA, LgA, HD, TH, IntA-HD, or IntA-FR—see below) for a 2-week access period after which Pmax was evaluated using a within-session TH procedure.

IntA-HD Group

Following acquisition, rats ($N=6$) were given access to cocaine using a HD procedure as previously described (Morgan *et al*, 2009). Briefly, the subjects were provided access to a lever which, when depressed, activated an infusion pump until the lever was released. An LED stimulus light above the lever was illuminated when the lever was depressed; that is, the light and pump were activated–inactivated concurrently. The dose self-administered was determined by the length of time the animal held the lever down (infusion rate was 0.375 mg/kg/s) and by the pattern

of responding. There were no timeout periods during these HD training sessions. The concentration of cocaine was 5 mg/ml. Rats were given daily 3-h sessions until the response pattern stabilized (which generally took 2–3 days). After they had acquired the HD response, the schedule was switched to an IntA schedule in which subjects were given access to the retractable HD lever for twelve 5-min access periods on the HD schedule as previously described (Zimmer *et al*, 2011). In between each 5-min trial, a 25-min timeout period was enforced during which the lever was retracted. The lever presence–absence served as the only signal for drug availability. Animals received daily IntA-HD sessions for 14 consecutive days.

IntA-FR Group

A separate group ($N=5$) was tested using the same IntA procedure described above (twelve 5-min trials separated by 25-min timeout periods) except that, instead of using a HD procedure, responding was reinforced on an FR1 schedule. The concentration of cocaine was 5 mg/ml. Each response produced a 1-s infusion of drug (0.375 mg/kg/inf) signaled by the illumination of a 1-s presentation of an LED stimulus light. Apart from the 1-s interval during which the pump was active, no timeouts were imposed to limit the number or timing of infusions within the 5-min access periods. This procedure lies midway in the continuum of control of drug dose and injection speed with the HD procedure on the one extreme and a fixed unit dose on the other. That is, with a small unit dose and no timeout it is possible for an animal to self-administer, for example, 4–5 small injections in an 8- to 10-s interval. Pilot studies have shown that the patterns of intake with this procedure are similar to those observed on the HD schedule.

LgA Group

Subjects in the LgA group ($N=9$) were given access to cocaine (0.75 mg/kg; infused over 4 s) on an FR1 schedule during daily 6-h sessions for 14 consecutive days. At the start of each infusion, a stimulus light signaled a 20-s timeout period during which the lever was retracted.

ShA Group

One group ($N=6$) was given access to cocaine (0.75 mg/kg; infused over 4 s) on an FR1 schedule during 2-h daily sessions for 14 consecutive days. At the start of each infusion, a stimulus light signaled a 20-s timeout period during which the lever was retracted.

HD Group

Subjects ($N=7$) in this group self-administered for 14 daily, 2-h sessions on the HD schedule of reinforcement as described by Morgan *et al* (2009). Briefly, the syringe-pump became active when the lever was depressed and became inactive when the lever was released. The size and speed of each dose was determined by the duration and spacing of HD responses (infusion rate was 0.375 mg/kg/s). An LED stimulus light above the lever was illuminated when the lever was depressed; that is, the light and pump were activated–inactivated concurrently. No timeout periods were imposed to limit the size or number of doses self-administered.

Within-Session TH Group

Subjects in the TH group ($N=5$) were given access to cocaine for 14 consecutive days on the within-session TH procedure (Oleson *et al*, 2011). Rats were given access to a descending series of 12 unit doses of cocaine (421, 237, 133, 75, 41, 24, 13, 7.5, 4.1, 2.4, 1.3, and 0 μg/injection) on an FR1 schedule during consecutive 10-min bins within a 2-h daily session. An LED stimulus light signaled the duration of the infusion and the corresponding time-out period (ie, equal to the pump duration). The lever was not retracted at any time during the session. Doses were manipulated by holding the concentration constant and adjusting the pump duration (see supplementary material of Oleson and Roberts (2009) for a full characterization of this approach and validation that the appropriate quantity of drug is delivered across all pump durations). Each TH session lasted 2 h. The TH group was exposed to these conditions during the 14-day test period. All groups, including the TH group, self-administered on this schedule following their 14-day test period for 3 consecutive days.

Data Analysis

A behavioral economic analysis was used to quantify results from this procedure. Behavioral economic theory has been successfully applied to drug self-administration in general (Bickel *et al*, 1993; Hursh, 1991) and TH procedures in particular (Espana *et al*, 2010; Oleson *et al*, 2011; Oleson and Roberts, 2009). In the current TH procedure, the descending series of doses (listed above) resulted in rats receiving access to cocaine across the following 11 ascending unit-prices: 2.4, 4.2, 7.5, 13.3, 23.7, 39.9, 75, 134, 242, 417, and 750 responses/mg. The primary dependent measure analyzed was the maximal price paid for cocaine (Pmax), which was determined to be the unit-price corresponding to the apex of the price-response function as previously described (Espana *et al*, 2010; Oleson *et al*, 2011). An example of the calculation of Pmax is shown in Figure 4a. The dose that maintains the highest rate of responding is defined here as the TH dose. This can be converted to Pmax by calculating the responses required at the observed TH dose to obtain 1 mg cocaine. For example, if the TH dose were found to be 7.5 μg/inj, then the Pmax would be 133.3 responses/1 mg cocaine. In the vocabulary of behavioral economics, Pmax coincides with the point at which cocaine consumption changes from being maintained (inelastic demand) to not being maintained (elastic demand). Pmax was calculated from data averaged across 3 consecutive days of TH test sessions.

The TH procedure affords the opportunity to assess both appetitive and consummatory responding (as discussed in Oleson *et al*, 2011). Drug intake measured during early phases (10–40 min) of the TH procedure yields a measure of consummatory responding relatively unconstrained by price. Mean intake during 10–40 min were calculated for each animal. Intake was calculated in the early stage of the TH procedure because this is a period when the price of

cocaine is relatively inexpensive and therefore less likely to constrain the animal's intake. The first 10 min were excluded to avoid the loading phase of the session.

Brain-Cocaine Concentration Model

Brain-cocaine concentrations were calculated as previously described (Zimmer *et al*, 2011) using equations employed by Pan *et al* (1991). Briefly, the equation

$$c = \frac{dk}{v(\alpha - \beta)}(e^{-\beta t} - e^{-\alpha t})$$

estimates the amount of cocaine in the brain compartment at time t. This equation accounts for the dose of cocaine (d), the transfer of cocaine between the blood and the brain ($k = 0.233\ \text{min}^{-1}$), the apparent brain volume ($v = 0.151\ \text{kg}^{-1}$), and the removal of cocaine from the blood through redistribution ($\alpha = 0.642\ \text{min}^{-1}$) and elimination ($\beta = 0.097\ \text{min}^{-1}$).

Drugs

Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, NC), was dissolved in a solution of sterilized saline 0.9% and passed through a microfilter (0.45 μm pore size). A 5 mg/ml cocaine solution was used for HD, within-session TH, and IntA experiments; a 2.5 mg/ml solution was used for acquisition, ShA and LgA.

RESULTS

Figure 1 illustrates representative response patterns generated by each of the six procedures and the corresponding modeled brain levels. Each panel consists of the mathematically modeled brain-cocaine concentration (left axis) and the cumulative dose (right axis) self-administered throughout the session. Animals self-administering on an FR1 schedule (ShA and LgA) titrated their brain levels of cocaine by regularly spacing their responses (Figures 1a and b). The HD group displayed a steady rate of responding as previously described (Morgan *et al*, 2009). This led to a relatively stable level of cocaine throughout the session (Figure 1c). The within-session TH group also showed a stable level of cocaine only during the first part of the session with brain levels of cocaine falling as the price of cocaine increased beyond the animal's Pmax (Figure 1e). The IntA animals had access to cocaine on either an FR1 (0.375 mg/kg/inf) or HD procedure during 5-min trials

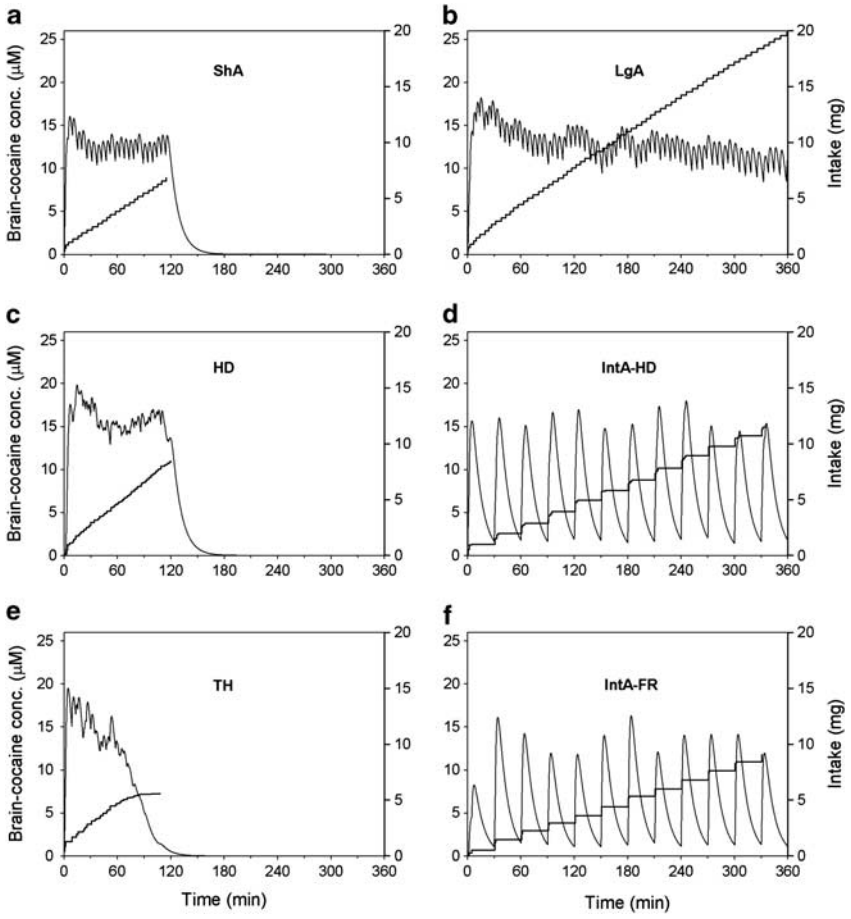

Figure 1 Intake and modeled brain levels of cocaine for representative animals tested using six distinct self-administration procedures. Each panel shows the modeled brain levels of cocaine (left axis) and cumulative intake (right axis) throughout a session for an individual rat self-administering for cocaine on a ShA (a), LgA (b), HD (c), IntA-HD (d), TH (e), or IntA-FR (f) procedure.

followed by a 25-min timeout period. This pattern of access led to large fluctuations of brain-cocaine concentrations throughout the 6-h session (Figures 1d and f). The pattern of intake was virtually identical between the IntA-HD and the IntA-FR groups. Subjects were observed to self-administer large doses (>3.0 mg/kg) mostly in the first minute of the trial as previously described (Zimmer *et al*, 2011). It is important to note that the IntA-FR animals had no timeouts within their 5-min access period allowing them to self-administer clusters of injections at the beginning of the access period in a similar manner as the IntA-HD group.

Figure 2a illustrates the mean daily intake for each group during the 2-week test period. The data failed a test for homogeneity of variance and normality and were therefore transformed to rank orders. A two-way repeated-measures analysis of variance (ANOVA) revealed a significant DAYS effect ($F(13, 406) = 4.19$, $p < 0.001$), and measures of the LgA group intake showed a 21.8% increase. No significant GROUP × DAYS interaction was observed. The average intake per session for each group is shown in Figure 2b. A significant GROUP effect was observed ($F(5, 32) = 98.19$, $p < 0.001$) and a Holm–Sidak *post-hoc* test revealed a significant difference of the LgA group compared with each of the other groups. No other comparisons were statistically significant.

The average number of responses per session is shown in Figure 2c. The data set failed a test of homogeneity of variance, therefore a Kruskal–Wallis *H*-test was conducted instead of an ANOVA. A statistically significant GROUP effect was observed ($H\ (5) = 31.73$, $p < 0.001$). A Dunns multiple comparison procedure revealed that the animals in the TH, HD, and IntA-HD groups responded more than ShA animals. The TH and HD groups also differed from the IntA-FR group.

Following the 2-week test period, during which each group self-administered on their respective schedules, all animals were tested using a within-session TH procedure. Figure 3 shows representative event records for a ShA (top) and an IntA-HD subject (bottom). This comparison illustrates how animals might have similar rates of responding in the early part of the session but cease responding at very different times (corresponding to different doses). The IntA-HD animal increased its response rate throughout the session compensating for the decrease in unit dose and continued responding into the eleventh bin, whereas the ShA animal ceased responding after the sixth bin. Mean (±SEM) responses during each 10-min bin for every group are shown (Figure 3, bottom) illustrating the differences between groups in response rate during the TH schedule. These differences in intake were subjected to a behavioral economic analysis.

Figure 4 shows the behavioral economic analysis of data derived from the TH procedure. An example session is shown in Figure 4a to illustrate the method used to determine Pmax. Responses (closed circles) and intake (open circles) are graphed during the 120-min session. Note that the animal in this example maintained a stable intake during the first 80 min of the session by increasing the response output in each consecutive bin. In bin 8, the highest number of responses is observed, and all subsequent bins were marked by a failure to maintain stable cocaine intake. This inflection point (Pmax—dotted line) was determined for each animal. Figure 4b shows the average (±SEM) Pmax for each group. As the Pmax values were derived from an exponential series of doses, a log transform was

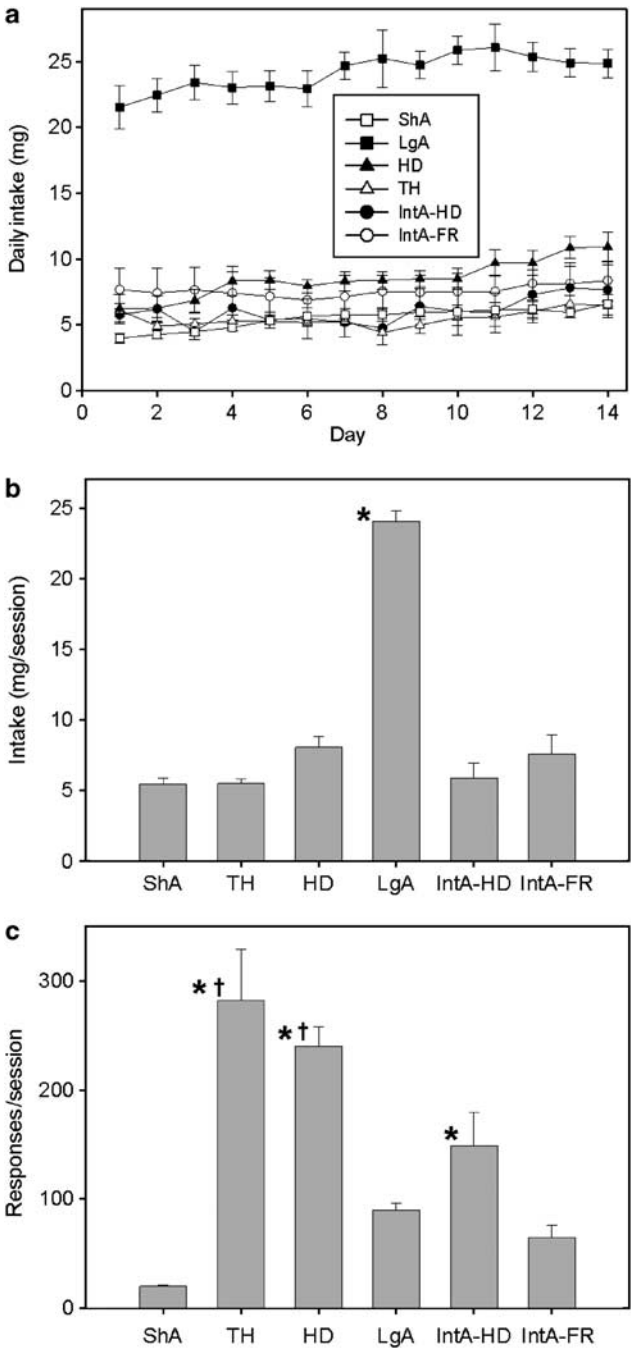

Figure 2 Average intake and responses during the 2-week test period. Panel (a) shows the daily intake self-administered for each day over the 14-day test period for each group. Each symbol represents the mean intake self-administered for that group (±SEM). Panel (b) shows the average (±SEM) intake per session for each group. LgA animals self-administered significantly more cocaine than all other groups ($p < 0.001$). Panel (c) shows the responses per session for each group. Bar represents the mean (±SEM) number of responses per session. The TH, HD, and IntA-HD groups responded significantly more than ShA animals ($^{*}p < 0.05$), and both TH and HD groups responded more than the IntA-FR group ($^{\dagger}p < 0.05$).

performed on all data before ANOVA. This transform was necessary in order to meet the requirement of homogeneity of variance.

A two part analysis was performed. The first addressed the various control conditions. The TH and HD control groups were compared with the ShA group in order to test whether the HD response pattern and/or the high rate of responding engendered by the TH and HD procedures had an effect. No significant difference was revealed by a one-way ANOVA ($F(2, 17) = 1.37$, $p = 0.28$). No statistically significant difference was observed between the IntA-HD and IntA-FR groups (mean Pmax = 480.6 ± 114.7 and 480.1 ± 96.2, respectively) and data from these two groups were combined in the subsequent analysis. The central hypothesis that spiking blood levels would have a greater impact on Pmax relative to LgA and ShA was tested using a one-way ANOVA, which revealed a significant difference ($F(2, 25) = 13.07$, $p < 0.001$) between groups. A Holm–Sidak *post-hoc* analysis demonstrated that LgA and IntA animals had higher Pmax values than ShA, and IntA animals had higher Pmax values than LgA animals.

Drug intake measured during early phases (10–40 min) of the TH procedure yields a measure of consummatory responding relatively unconstrained by price. A two part analysis was performed on the groups as described above. A one-way ANOVA ($F(2, 17) < 1$) on the 3 control groups (ShA, TH, and HD) revealed no significant differences in consumption during the early phase of the TH procedure. Similarly, a one-way ANOVA ($F(2, 25) < 1$) revealed no significant differences between the main test groups (ShA, LgA, and IntA).

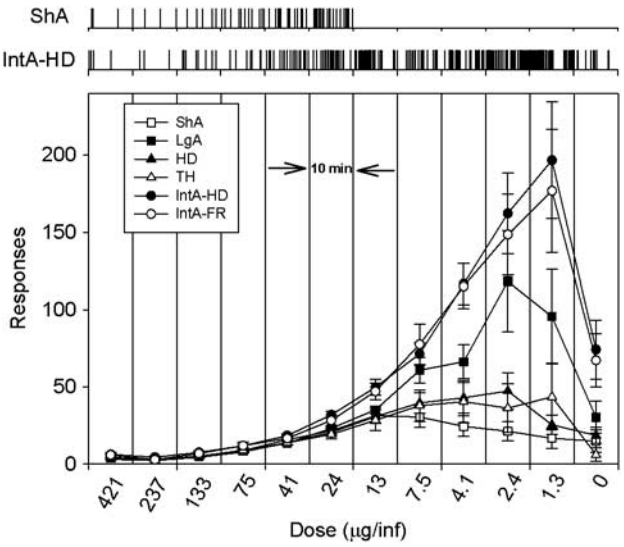

Figure 3 Response patterns and intake levels during the within-session TH procedure. Top: representative event records of animals responding on the TH schedule are shown for a representative ShA and IntA-HD animal. Note that the IntA-HD animal continued responding much longer into the session despite the increase response rate required to maintain preferred brain levels of cocaine. Bottom: average (± SEM) number of responses during each 10-min bin for each group is plotted. The apparent differences in these groups prompted a behavioral economic analysis (see Figure 4).

DISCUSSION

The present experiment was designed to test the hypothesis that IntA is sufficient to increase Pmax values, a behavioral economic measure of the motivation to self-administer drug (Bickel *et al*, 1993; Hursh and Winger, 1995; Oleson *et al*, 2011; Oleson and Roberts, 2009). Six groups with histories of different self-administration procedures, which produced very different patterns of drug intake and drug dynamics, were compared. Combined data from the two IntA groups (IntA-HD and IntA-FR) showed robust increases in Pmax relative to both ShA and LgA animals. These groups received 12 opportunities to self-administer each day during 5-min trials separated by 25-min timeouts. Rats in these groups often self-administered large doses of cocaine (eg, >3.0 mg/kg)

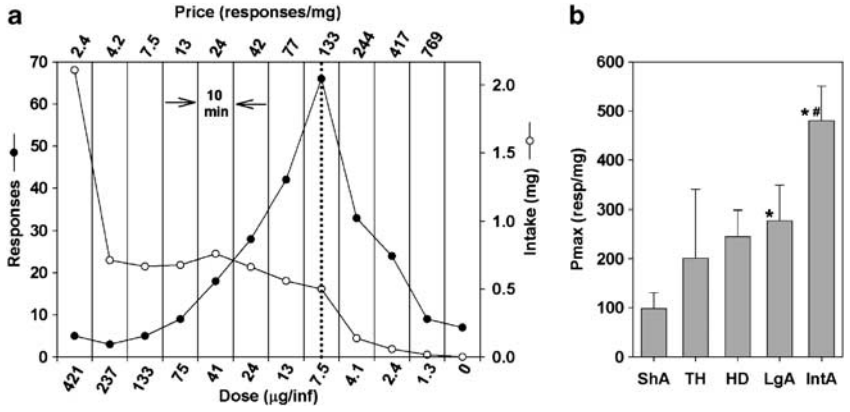

Figure 4 Behavioral economic analysis of data obtained from the within-session TH procedure. Panel (a) shows a representative animal responding during the TH schedule. Closed circles represent the number of responses emitted during each 10-min bin. Note that the unit dose of cocaine decreased during each bin leading to an increase in responses. Open circles show the intake of cocaine during each bin, demonstrating that total intake of cocaine remained relatively stable through the first 80 min of the schedule despite the rising price of cocaine. The dotted line represents the inflection point (Pmax) at which the animal failed to increase responding to maintain a stable level of intake. The Pmax value was calculated for all animals as the price (responses/mg of cocaine) animals reached before responding dropped off. Average (± SEM) Pmax values are plotted for each group in panel (b). Values statistically higher ($p < 0.05$) than the ShA are denoted by an (*), and values higher than the LgA group are denoted by a pound sign (#).

usually in the beginning of the trial and in a relatively short period of time (~60 s). This pattern is consistent with our previous report (Zimmer *et al*, 2011) showing that rats rapidly self-administer large doses of drug when blood levels have been forced to low levels. These results suggest that rapid spiking of brain-cocaine levels is sufficient to increase Pmax.

The application of a well-established mathematical model for estimating cocaine levels in brain (Ahmed and Koob, 2005; Nicola and Deadwyler, 2000; Pan *et al*, 1991; Wise *et al*, 1995) helps illustrate the important differences between the groups during daily self-administration sessions. Brain concentrations of cocaine rose relatively rapidly in all groups at the beginning of the session. This well-characterized phenomenon has been referred to as a loading phase (Wilson *et al*, 1971), and is typically followed by a maintenance phase during which drug levels are maintained within relatively narrow limits. In this study, four groups were allowed to progress into the maintenance phase for varying lengths of time. The TH group maintained blood levels for only 60–90 min (depending on individual Pmax), the ShA and HD groups maintained blood levels for 2 h, and the LgA group for 6 h. By contrast, the two IntA groups were exposed to a very different drug-level dynamic—that is, 12 distinct cocaine spikes within each 6-h session. This spiking pattern of intake was associated with the highest Pmax values. Note that as the IntA procedures do not allow blood levels to be maintained, it would appear that sustained blood levels are not necessary and that spiking blood levels are sufficient to increase the maximum price paid for cocaine.

The hypothesis tested here that spiking cocaine levels might be important to the addiction process was prompted by both preclinical and clinical data. On the clinical side, textbooks and the research literature emphasize the importance of the 'rush' or intense subjective effects, which are mediated by larger doses and faster routes of administration (Seecof and Tennant, 1986; Kumor *et al*, 1989; Gorelick, 2009; Volkow and Li, 2009). Presumably, these intense intoxicating events increase the probability of future use. Over time, individual or grouped intoxicating events can progress to a cyclical binge-abstinent style of intake (Gawin, 1991). The idea that 'binge-like' intake is an important part of the addiction process has prompted the development of rodent models that allow for daily 'binges' to occur, such as in the LgA procedure (Ahmed and Koob, 1998). What has been missing from the clinical literature is information on the pattern of drug use within a binge. A recent survey of experienced cocaine users shows the interval between uses within a cocaine binge might be, on average, well over an hour (Beveridge *et al*, 2012). Given the short half-life of cocaine (~40 min; Javaid *et al*, 1983) in humans, these survey data suggest that blood levels are not maintained throughout the binge period but may decrease substantially between each intoxicating event. Although more clinical data on dose size and inter-use interval within a binge would be helpful, it appears that fast-rising and subsequent decline in blood levels is a pattern that can lead individuals down the addiction cycle.

The spiking cocaine-level hypothesis was also suggested by recent preclinical data. A self-administration procedure that produces spiking brain levels in rats was developed in our lab while we were investigating the relationship between the preferred dose of cocaine and drug levels in the brain (Zimmer *et al*, 2011). Animals were given access to drug during 5-min trials, and, in order to force a decline in blood levels, the trials were separated by timeout periods ranging from 10 to 25 min. The primary finding of the experiment was that the selected dose of cocaine was inversely related to blood levels. That is, rats selected relatively low doses of drug when blood levels were high and selected very large doses (~3 mg/kg/inj) of cocaine when blood levels were low. After completion of the experiment, animals were given access to cocaine on a progressive ratio (PR) schedule and were found to have markedly elevated breakpoints (unpublished). Although we had no control group to compare results with, it appeared that animals that had experienced a spiking pattern of cocaine intake showed much higher breakpoints than was typically seen in our lab. This study represents an attempt to quantify the increases in motivation we observed relative to groups that self-administered cocaine with varying drug dynamics.

A within-session TH procedure was chosen to compare the effects of the different cocaine self-administration histories. This procedure was adapted from a between-session TH procedure in which a series of doses were tested on consecutive days (Oleson and Roberts, 2009; Zittel-Lazarini *et al*, 2007). The procedure involves giving access to cocaine on an FR1 schedule at a fixed unit dose, which is reduced through a series of 11 doses every 10 min (see Materials and methods section). A behavioral economic analysis of the response rate and drug intake at each interval yields measures of both consumption and maximum price paid and thus affords the opportunity to investigate appetitive and consummatory aspects of self-administration within the same procedure. Consummatory behavior is reflected as cocaine intake early in the session (at high unit doses) when the response cost is low. Appetitive behavior is assessed later in the session by determining the lowest unit dose (ie, highest unit price) that maintains consumption. Typically in self-administration studies, price is manipulated by holding a drug dose constant and increasing the response requirement (Cosgrove and Carroll, 2002; Wade-Galuska *et al*, 2007); however, fixing the response requirement and decreasing the available unit dose accomplishes the same thing (Bickel *et al*, 1990). Here, the decreasing series of unit doses resulted in an ascending series of unit prices (2.4, 4.2, 7.5, 13, 24, 40, 77, 133, 244, 416, and 750 responses/mg). Pmax values are theoretically related to breakpoints derived from a PR schedule. Although the PR and TH procedures measure different aspects of appetitive behavior—work output to obtain a large bolus injection (PR) *vs* work output to maintain a constant blood level despite diminishing returns (TH)—there seems to be a high correlation between the two dependent measures. Indeed, several pharmacological manipulations that have been shown to have an effect on PR breakpoints such as haloperidol (Depoortere *et al*, 1993; Roberts *et al*, 1989) or baclofen (Roberts *et al*, 1996; Brebner *et al*, 2000) had a similar effect on TH Pmax (Oleson *et al*, 2011). The PR breakpoint and the TH Pmax thus permit detailed assessment of the relationship between work output and obtained reinforcement and

together may be particularly helpful in characterizing changes in drug intake produced by prolonged drug exposure (Vezina, 2004).

In the present experiments, spiking brain levels were produced using an IntA procedure with two different response requirements. The HD response was first described by Morgan *et al* (2009) as an attempt to bypass unit dose and give the animal more control over the size and speed of injections. Animals appeared to titrate their blood levels within a narrow range and adjusted their response pattern to compensate changes across a 16-fold range of cocaine concentrations (Morgan *et al*, 2009). For comparison, we also included an IntA group reinforced under an FR1 schedule during the 5-min access periods. It is important to note that no timeout was used with the FR1. Thus, animals were permitted to self-administer multiple injections (0.375 mg/kg/inf) within a few seconds. The IntA-FR and IntA-HD groups showed similar intakes during the 5-min trials and across sessions. We found no observable differences in intake or Pmax values between these two groups. Comparing the two IntA groups with controls also allowed us to rule out the possibility that the increase in Pmax was produced by overall cocaine intake (Figure 2b) or by high rates of responding (Figure 2c). Therefore, the increases in Pmax observed in IntA animals are likely due to the spiking brain levels of cocaine throughout the session. Although the IntA-HD group offers some unique advantages in terms of data analysis (eg, selected dose size, speed), the IntA-FR schedule represents an easier method of generating the phenotype reported in this study.

The LgA procedure represents the best current model of escalation of intake. Animals in this paradigm reliably show increases in intake of 30–40% over the course of 2 weeks (Ahmed and Koob, 1998). In most cases, this procedure has resulted in an increase in motivational measures such as PR breakpoints (Paterson and Markou, 2003; Wee *et al*, 2008; but see Liu *et al*, 2005a; Quadros and Miczek, 2009) and drug-induced reinstatement responding (Mantsch *et al*, 2008). The effect of LgA on Pmax appears to depend on procedural variables. We previously observed a decrease in Pmax in LgA animals using a between-session TH procedure (Oleson and Roberts, 2009). In that procedure, the dose of cocaine decreased each day over the course of 11 days. In this study, the entire dose-effect curve was evaluated within a single session, and the LgA group showed an increase in Pmax. These two TH procedures address different aspects of the motivation to self-administer cocaine. The within-session TH procedure allows animals to take large loading doses of cocaine when the price is relatively inexpensive; the price then increases through the session. Pmax in this case appears to measure the maximal price a rat will pay to maintain brain levels of cocaine. By contrast, the between-session TH procedure effectively measures the maximal price an animal will pay to load cocaine brain levels. It appears that LgA can, under some circumstances, produce an increase in some motivational measures.

Our conclusion, that spiking brain levels of cocaine produce an increase in the motivation to self-administer drug, depends on the accuracy of the mathematical model used to estimate brain-cocaine concentrations. The equation used by Pan *et al* (1991) is based on standard pharmacokinetic principles that have been validated for many different drugs and in many different systems (Karan *et al*, 2009). However, the specific variables used to represent rat blood volume, cocaine redistribution and degradation must be verified in order to have confidence in applying the equation to this study. Fortunately, use of this model has been widespread. Many self-administration studies have applied the equation to estimate brain concentrations of cocaine in live animals (Ahmed and Koob, 2005; Samaha *et al*, 2002; Zernig *et al*, 2007; Zimmer *et al*, 2011), and the equations have also been applied in studies using electrophysiology (Nicola and Deadwyler, 2000; Peoples and Cavanaugh, 2003; Peoples *et al*, 2004, 2007), microdialysis (Wise *et al*, 1995) and voltammetry (Hermans *et al*, 2008; Stuber *et al*, 2005a, b). These studies have demonstrated that the modeled brain-cocaine concentrations are highly correlated with NAc dopamine levels (Hermans *et al*, 2008; Shou *et al*, 2006; Wise *et al*, 1995) as well as cocaine-induced locomotor behavior (Shou *et al*, 2006).

An extensive literature has demonstrated that continuous *vs* intermittent administration of psychostimulant drugs produces very different consequences on behavior and neurochemistry (for review, see Robinson and Berridge, 2008). For example, it has been shown that daily injections of cocaine induce long-lasting behavioral sensitization whereas continuous (minipump) administration produces behavioral tolerance (Reith *et al*, 1987; King *et al*, 1992). Differences in neurochemistry have also been shown, such as subsensitivity or supersensitivity of the D2 autoreceptor following intermittent or continuous administration of cocaine respectively (Jones *et al*, 1996). Intermittency typically refers to daily or every-other-day administration of drug; however, the present data suggest that the theoretical intermittency/continuous distinction might reasonably apply to 6-h cocaine self-administration sessions. Given that a 25-min timeout period allows for a $\sim$90% clearance of cocaine, the twelve 5-min access periods used here would constitute an intermittent dosing regimen; a 6-h LgA session would more closely represent continuous drug delivery. It has been suggested that continuous administration is the better model of a human binge (eg, King *et al*, 1994) based on the assumption that addicts self-administer at frequencies that result in sustained cocaine levels. However, recent clinical studies have challenged this assumption (Beveridge *et al*, 2012; see Introduction section). In fact, the pattern of human intake may more closely resemble intermittent administration. Future studies will be necessary to fully characterize the role of intermittency on the transition to drug addiction as well as the underlying neurobiological mechanisms involved.

Here, we report that the schedules that induce both fast rise-times and large fluctuations in brain-cocaine concentrations produce the most robust increases in motivation to self-administer cocaine. The observed increases in Pmax could not be accounted for by intake, contingency or rates of responding. Measures of Pmax were higher in IntA animals than LgA animals, indicating that when it comes to producing an increase in motivation the pattern of intake is likely more important than the total amount consumed.

ACKNOWLEDGEMENTS

This study was supported by the NIDA research Grants R01 DA14030 (DCSR), P50 DA06634 and R21 DA029405 (Freeman). The excellent technical assistance of Leanne N Thomas and Holly V Buben is gratefully acknowledged.

DISCLOSURE

The authors declare no conflict of interest.

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