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ORIGINAL INVESTIGATION

High and escalating levels of cocaine intake are dissociable from subsequent incentive motivation for the drug in rats

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Abstract
Rationale Taking high and increasing amounts of cocaine is thought to be necessary for the development of addiction. Consequently, a widely used animal model of drug self-administration involves giving animals continuous drug access during long sessions (LgA), as this produces high and escalating levels of intake. However, human cocaine addicts likely use the drug with an intermittent rather than continuous pattern, producing spiking brain cocaine levels.
Objectives Using an intermittent-access (IntA) cocaine self-administration procedure in rats, we studied the relationship between escalation of cocaine intake and later incentive motivation for the drug, as measured by responding under a progressive ratio schedule of cocaine reinforcement.
Results First, under IntA, rats escalated their cocaine use both within and between sessions. However, escalation did not predict later incentive motivation for the drug. Second, incentive motivation for cocaine was similar in IntA-rats limited to low- and non-escalating levels of drug intake (IntA-Lim) and in IntA-rats that took high and escalating levels of drug. Finally, IntA-Lim rats took much less cocaine than rats given continuous drug access during each self-administration session (LgA-rats). However, IntA-Lim rats later responded more for cocaine under a progressive ratio schedule of reinforcement.
Conclusions Taking large and escalating quantities of cocaine does not appear necessary to increase incentive motivation for the drug. Taking cocaine in an intermittent pattern—even in small amounts—is more effective in producing this addiction-relevant change. Thus, beyond the amount of drug taken, the temporal kinetics of drug use predict change in drug use over time.

Keywords Addiction · Intravenous drug self-administration · Intermittent access · Long access · Escalation of cocaine intake · Binge-like cocaine intake · Progressive ratio

Introduction

High and escalating levels of drug intake are thought to be critical in inducing symptoms of addiction, in particular to cocaine (Ahmed 2012; Edwards and Koob 2013). As such, escalation of drug intake is a major focus in cocaine self-administration research. To model high and escalating levels of drug intake, animals are given continuous drug access during long sessions, typically lasting 6 h [long-access, LgA; (Ahmed and Koob 1998)]. This promotes cognitive changes and addiction-like symptoms compared to shorter self-administration sessions [1–2 h; (Ahmed and Koob 1998, 1999; Bouayad-Gervais et al. 2014; Briand et al. 2008; George et al. 2008; Hao et al. 2010; Knackstedt and Kalivas 2007; Paterson and Markou 2003; Vanderschuren and Everitt 2004)]. LgA drug self-administration is considered the current gold standard for studying the neurobiology of addiction (Edwards and Koob 2013). The underlying assumption is that "excessive drug exposure likely remains an indispensable element driving the development of addiction" (Edwards and Koob 2013) and that "[…] below this critical level of exposure, there would be no drug-induced neuropathological

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changes, and drug use would remain under control, at least in the majority of drug-exposed animals" (Ahmed 2012).

An important question is how well the LgA-procedure models how human addicts take cocaine. By necessity but also by choice, cocaine users adopt intermittent patterns of use both within and between periods of consumption [reviewed in Allain et al. (2015)]. Experienced cocaine users typically engage in recurring binges, rather than continuous daily use (Gawin and Kleber 1986), and bouts of intoxication are interspersed with periods of abstinence, often used to gather money for the next dose (Simon et al. 2002; Ward et al. 1997). Cocaine intake might also be intermittent *within* a bout of intoxication. Experienced (25–32 years of use) and less experienced (4–9 years of use) cocaine users take their cocaine in the same amount of time. But experienced users take their purchased drug in fewer intervals. This would presumably produce more pronounced "spikes" and "troughs" in cocaine concentrations in the blood/brain (Beveridge et al. 2012).

To model this intermittent pattern of cocaine use, Zimmer et al. (2011) developed an intermittent-access (IntA) self-administration procedure in rats; cocaine is available during 5-min periods intercalated with 25-min periods during which drug is not available (Zimmer et al. 2011). In contrast to LgA-procedures which achieve high and sustained brain levels of drug, IntA models the intermittent spikes in brain cocaine levels (Zimmer et al. 2011; Zimmer et al. 2012) that are thought to be clinically relevant (Beveridge et al. 2012). The IntA-procedure also produces addiction symptoms more effectively. LgA-rats consume much more drug but IntA-rats develop greater incentive motivation for cocaine (Zimmer et al. 2012). IntA-rats also show sensitization to cocaine's effects at the dopamine transporter, while LgA-rats show tolerance (Calipari et al. 2013). This sensitization effect can be seen after as little as three IntA self-administration sessions and it is linked to an increase in the motivation to take cocaine (Calipari et al. 2015; Siciliano and Jones 2017). Finally, IntA-rats show robust psychomotor sensitization (Allain et al. 2017)—an addiction-relevant change difficult to measure with LgA [(Ahmed and Cador 2006; Ben-Shahar et al. 2004; Knackstedt and Kalivas 2007) but see (Ferrario et al. 2005)].

Similar to LgA, IntA can also promote escalation of drug use over sessions (Kawa et al. 2016; Pitchers et al. 2017). But is escalation necessary to produce the brain changes that push the addiction process forward, even under IntA-conditions? Here, we determined how escalation of cocaine intake during IntA-experience influences later incentive motivation for the drug. First, we determined whether escalation of cocaine use during IntA-sessions promotes increased incentive motivation for the drug. Second, we compared incentive motivation for cocaine following LgA-experience with escalation versus following IntA-experience without escalation.

Materials and methods

Subjects and apparatus

Male Wistar rats (225–250 g; Charles River Laboratories, St Constant, QC) were housed individually in a climate-controlled room under a reverse 12-h light/dark cycle (Lights off at 08:30 a.m.). Only male rats were studied in this series of experiments because the IntA model of cocaine self-administration is new (Zimmer et al. 2011), and it has not yet been characterized in female animals. However, there are sex differences in the response to drugs of abuse (Becker 2016). This requires that both males and females be represented in animal models. To this end, we are currently examining potential sex differences in cocaine self-administration behavior under IntA conditions.

Water was available ad libitum and food was restricted to 25 g/day. Rats were tested in standard operant conditioning cages (Med Associates, St Albans, VT). These were equipped with a house light, a food pellet dispenser and receptacle, a drug infusion line for intravenous (i.v.) injections, two retractable levers, and a discrete light above each lever. Pressing the active lever was reinforced with either a food pellet or intravenous (i.v.) cocaine, as detailed below. Pressing the inactive lever had no programmed consequences. To signal the beginning of each test session, the two levers were inserted into the cage and the house light was illuminated. Upon reward delivery (and during the timeout period where applicable), the light above the active lever was illuminated and both levers were retracted. The light was then extinguished and the levers were again inserted into the cage to indicate reward availability. The Université de Montréal's animal care committee approved all experimental procedures, and these followed the guidelines of the Canadian Council on Animal Care.

Operant responding for food and surgery

Figure 1 shows the sequence of experimental events. To facilitate the acquisition of lever-pressing behavior and thus reduce the time needed to subsequently learn to self-administer cocaine, rats were first trained to press a lever for 45-mg, banana-flavored, grain-based food pellets (VWR, Town of Mount-Royal, QC), under a fixed ratio 1 schedule of reinforcement (FR1). Food training sessions lasted 30 min or until 100 pellets were self-administered. On the day following acquisition of this behavior (as indicated by the self-administration of ~ 25 pellets/session, on two consecutive sessions), rats were implanted with a catheter into the jugular vein (Samaha et al. 2011; Weeks 1962). To avoid blood clots in the catheters, catheters were flushed with saline or with saline containing 0.2 mg/ml of heparin (Sigma-Aldrich, Oakville, ON) and 2 mg/ml of enrofloxacin (CDMV, St

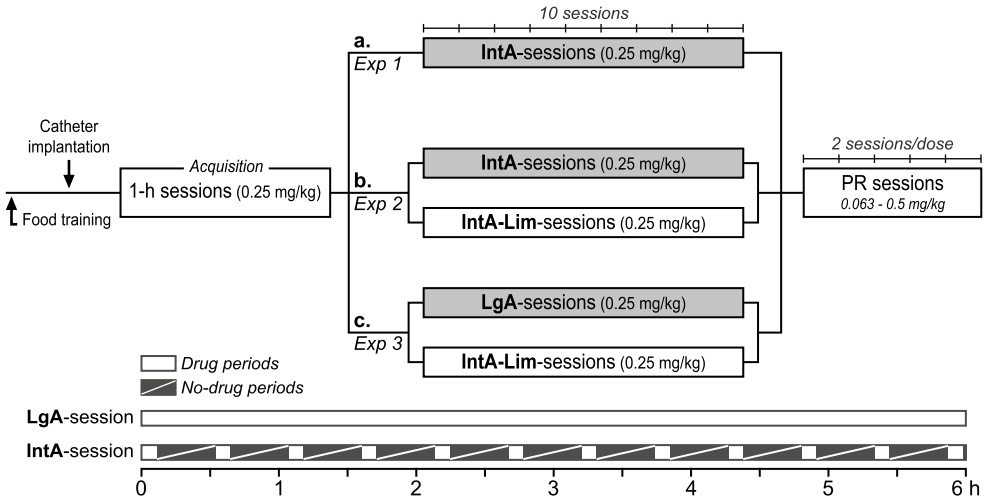

Fig. 1 The sequence of experimental events. In Experiment 1 a., rats self-administered cocaine intermittently (IntA) for 10 sessions. In Experiment 2 b., two groups of rats self-administered cocaine intermittently. One group was given IntA-sessions, during which drug access was unlimited during each 6-min cocaine period (referred to as the "IntA-unLim" group in the text). This permits escalation of drug intake. The other group was given IntA-Lim sessions, where drug access was limited to four injections/6-min cocaine period. This precludes escalation of drug intake. In Experiment 3 c., one group was given long-access sessions during which access to cocaine was continuous (LgA-rats). A second group was given IntA-sessions during which cocaine access was limited to two injections/6-min drug period (IntA-Lim rats). At the end of each experiment, breakpoints maintained by cocaine were assessed under a progressive ratio schedule of reinforcement (PR). Independent cohorts of rats were used in each experiment

Hyacinthe, QC) on alternate days. Rats were given at least 5 days of recovery prior to additional behavioral testing.

Acquisition of cocaine self-administration behavior

During 1-h sessions, each active-lever press produced an injection of cocaine hydrochloride (0.25 mg/kg/injection, over 5 s; Medisca Pharmaceutique, St Laurent, QC) and a 20-s timeout period. Once rats had a regular pattern of intake on two consecutive sessions and took at least six injections/session, the timeout period was removed for three final 1-h sessions. Rats were then given ten 6-h self-administration sessions, under IntA or LgA conditions. At the end of each experiment, catheter patency was verified by i.v. injection of 0.2 ml of the short-acting barbiturate, sodium thiopental (20 mg/ml in sterile water; CDMV, St Hyacinthe, QC). All rats in all experiments became ataxic within 10 s of this injection. Only one rat was excluded in Experiment 3 (IntA-Lim rat), for aggressive behavior.

IntA-sessions

Rats received one IntA-session/day, for 10 days. Each IntA-session had twelve 6-min drug periods during which cocaine (0.25 mg/kg/injection) was available without timeout under FR1, intercalated with 26-min no-drug periods during which levers were retracted and cocaine was unavailable (see bottom of Fig. 1). The last 6-min drug period was followed by a 2-min no-drug period such that the session lasted 6 h. In some experimental groups (IntA-unLim rats), access to cocaine was unlimited during each 6-min drug period, and IntA-sessions always lasted 6 h. In other experimental groups (IntA-Lim rats), we wished to preclude escalation of cocaine intake over sessions. To this end, IntA-Lim rats were limited to either four (Experiment 2; Fig. 1b) or two (Experiment 3; Fig. 1c) cocaine injections per 6-min drug period. The 26-min no-drug periods were initiated as soon as the allotted injections were self-administered. Thus, IntA-sessions could last less than 6 h in IntA-Lim rats. In Experiment 3, IntA-Lim rats were limited to two instead of four injections/drug period [as used previously (Allain et al. 2017)]. This maximizes differences in intake between IntA-Lim rats and the other experimental group studied in Experiment 3 (LgA-rats).

LgA-sessions

Rats received one LgA-session/day, for 10 days. During each 6-h session, cocaine was available continuously save for an 85-s timeout period following each self-administered injection. The timeout period was imposed to protect animals from taking potentially health-threatening amounts of cocaine (Bozarth and Wise 1985; Fitch and Roberts 1993). Note that in Experiment 3 (Fig. 1c), in both LgA-rats and IntA-Lim rats, drug was available under a fixed ratio 3 schedule of reinforcement to increase discrimination between the active and inactive levers.

Cocaine self-administration under a progressive ratio schedule of reinforcement

One to 4 days following the last IntA- or LgA-session, incentive motivation for cocaine was assessed by determining breakpoint for the drug under progressive ratio (PR; 0.063–0.5 mg/kg/injection, in counterbalanced order with 2–3 sessions/dose; all rats received all doses). During PR-sessions, the number of lever presses needed to obtain each successive injection increased exponentially according to the following formula: $[5 \times e^{(\text{number of injection} \times 0.2)} - 5]$ (Richardson and Roberts 1996). Each PR-session ended when 1 h elapsed since the last injection, or after 5 h. The last ratio reached prior to this point is termed the breakpoint, and it is an index of incentive motivation for drug (Richardson and Roberts 1996). We also analyzed session duration in some experiments. Session duration is the total time animals spend in the PR task. This includes the hour elapsed since the last injection.

Modeling of brain cocaine concentrations

We estimated brain cocaine concentrations (μM) using self-administration data from representative animals from each experimental condition. Estimates were calculated using the following equation derived by Pan et al. (1991):

$$C = dA \cdot \left(e^{-\beta t} - e^{-\alpha t}\right) \text{ with } A = \frac{k}{v \cdot (\alpha - \beta)}$$

The equation integrates the dose of cocaine *(d*, 0.25 mg/kg/injection) and the time elapsed after each self-administered injection (*t* in minutes). All other constants are those reported in Pan et al. (1991) for cocaine-experienced rats and are described in our previous work (Allain et al. 2017). This mathematical model is well established and has been used to estimate brain concentrations of cocaine following self- and experimenter-administered i.v. drug injections (Allain et al. 2017; Calipari et al. 2014b; Martin-Garcia et al. 2014; Nicola and Deadwyler 2000; Samaha et al. 2002; Shou et al. 2006; Wise et al. 1995; Zimmer et al. 2011; Zimmer et al. 2012). Here, we first calculated brain cocaine concentration separately for each self-administered drug injection of the session and then summed across injections to calculate the final concentration as a function of time. We used a 5-s time resolution for all estimates. Dr. David C. S. Roberts generously provided the Python script used to model brain cocaine concentrations.

Statistical analysis

In Experiment 1, one-way repeated measure ANOVA was used to analyze changes in self-administration behavior within and between IntA-sessions as well as breakpoints as a function of cocaine dose. Pearson's correlation coefficient *r* was computed to assess the relationships between breakpoint for cocaine and either level of escalation or prior cumulative cocaine intake. In Experiments 2 and 3, mixed-model ANOVA was used to analyze group differences in cocaine intake (session as a within-subject variable), in breakpoint for cocaine, and in PR session duration (cocaine dose as a within-subject variable). Cumulative cocaine intake was compared between groups using unpaired *t* tests. Significant interactions or main effects (p values < 0.05) were followed by Bonferroni's multiple comparisons tests.

Results

Experiment 1

Compared to LgA-rats, IntA-rats take much less cocaine but will also escalate their intake between sessions and show high motivation to obtain the drug (Kawa et al. 2016; Pitchers et al. 2017). We had two objectives here: (i) characterize escalation of cocaine intake between and within IntA-sessions and (ii) determine whether the extent of escalation during IntA-experience predicts the later motivation to take cocaine. Figure 1a shows the sequence of events.

IntA promotes escalation of cocaine intake within and between self-administration sessions

Figure 2a shows a representative pattern of cocaine intake during an IntA-session and the corresponding estimated brain cocaine concentrations [also see Zimmer et al. (2011)]. Figure 2b shows the number of self-administered cocaine injections during each 6-min cocaine period for the 1st (white circles), 5th (gray circles), and 10th (black circles) IntA-sessions. Cocaine intake escalated within each IntA-session. This effect was observed on the 1st IntA-session ($F_{11,\ 143} = 2.88$, $p = 0.002$; Fig. 2b) and it persisted in subsequent sessions (IntA-session 5, $F_{11,\ 143} = 6.92$; IntA-session 10, $F_{11,\ 143} = 5.78$; all p values < 0.0001; Fig. 2b). As shown in Fig. 2c, rats also escalated their intake between sessions (main effect of session, $F_{9,\ 117} = 9.78$, $p < 0.0001$). From the 4th session on, rats took more cocaine than on the 1st session (all p values < 0.01). Finally, the rats increased their rate of cocaine intake between sessions as well, as indicated by a decrease in the inter-injection interval (main effect of session, $F_{9,\ 117} = 7.27$, $p < 0.0001$; Fig. 2d). From the 3rd session on, rats self-administered cocaine injections at shorter intervals than on the 1st session (all p values < 0.05).

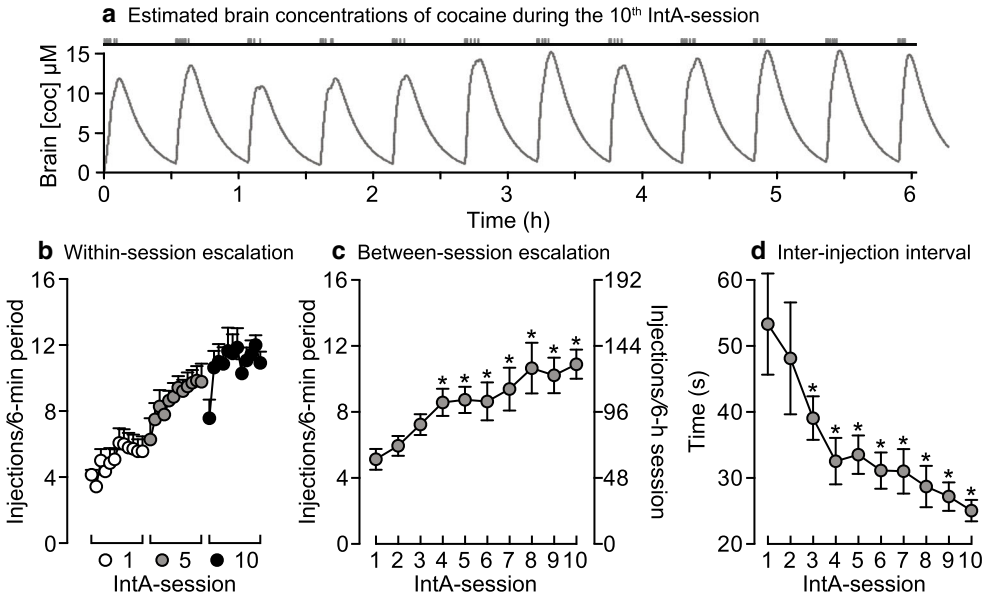

Fig. 2 Rats self-administering cocaine during intermittent-access sessions (IntA) escalate their drug intake within and between sessions. **a** Pattern of cocaine intake and estimated brain cocaine concentrations as a function of time during the 10th session, in a representative animal. **b** The number of cocaine injections taken during each of the 12 6-min cocaine periods within the 1st, 5th, and 10th IntA-sessions. In **c**, the left *Y*-axis shows the number of injections/6-min cocaine period and the right *Y*-axis shows the total number of injections/session. **d** The inter-injection interval [in seconds (s)] decreased significantly over sessions. *$p < 0.05$, vs. 1st IntA-session. Data are mean ± s.e.m. $n = 14$

IntA promotes a binge-like pattern of cocaine use

Figure 3a–c shows the pattern of cocaine intake in a representative animal during each of the twelve 6-min cocaine periods (divided in 90-s bins) of the 1st (Fig. 3a), 5th (Fig. 3b), and 10th IntA-sessions (Fig. 3c). Visual inspection of these data shows that cocaine intake was greatest at the beginning of each 6-min cocaine period (i.e., in the first 90 s) and that this drug-loading effect became more pronounced over sessions. Indeed, over the 10 IntA-sessions, rats increased the number of injections they took in the first 90 s of each 6-min cocaine period ($F_{9,\ 117} = 14.11$, $p < 0.0001$; Fig. 3d). From the 3rd session on, the number of these injections was greater than on the 1st session (all p values < 0.01). The number of cocaine injections taken in the subsequent 90-s bins also escalated over IntA-sessions ($F_{9,\ 117} = 2.74$; Fig. 3e; $F_{9,\ 117} = 2.05$; Fig. 3f; $F_{9,\ 117} = 2.72$; Fig. 3g; all p values < 0.05) but to a lesser extent than in the first 90-s bin (IntA-session × 90-s bin interaction effect, $F_{27,\ 351} = 9.3$, $p < 0.0001$; Fig. 3d–g). To explore this drug-loading effect further, we analyzed binge-like episodes during IntA-sessions [≥ 5 injections per 90-s bin, as adapted from Belin et al. (2009)]. Across IntA-sessions, a binge-like pattern of cocaine intake was seen in the first 90-s interval of each 6-min cocaine period (main effect of 90-s bin, $F_{3,\ 39} = 80.86$, $p < 0.0001$; Fig. 3h–k). The number of binge-like events during this interval also increased significantly between IntA-sessions ($F_{9,\ 117} = 13.44$, $p < 0.0001$; Fig. 3h). From the 3rd session on, there were more binge-like episodes than on the 1st session (all p values < 0.01; no other comparisons were statistically significant). Thus, rats "load up" on cocaine at the beginning of each 6-min cocaine period—taking many cocaine injections and at a rapid rate—and this effect increases across IntA-sessions.

Under IntA conditions, neither the extent of escalation nor cumulative cocaine intake predicts subsequent incentive motivation for the drug

Following the 10th IntA-session, all rats were given access to cocaine under a PR schedule of reinforcement and breakpoints maintained by the drug were measured. Rats reached higher breakpoints for greater doses of cocaine (main effect of dose, $F_{2,\ 26} = 17.16$, $p < 0.0001$; Fig. 4a). We performed a correlation analysis to determine how the level of escalation (difference between the number of cocaine injections taken on the 10th and 1st IntA-sessions) or cumulative cocaine intake (the total number of cocaine injections over the 10 IntA-sessions multiplied by 0.25 mg/kg/injection) might predict breakpoint for cocaine. Neither the level of escalation ($r^2 = 0.12$; Fig. 4b; $r^2 = 0.17$; Fig. 4c; $r^2 = 0.11$; Fig. 4d; all p values > 0.05) nor cumulative cocaine intake ($r^2 = 0.02$; Fig. 4e; $r^2 = 0.02$; Fig. 4f; $r^2 = 0.11$; Fig. 4g; all p values > 0.05) significantly predicted breakpoint for cocaine.

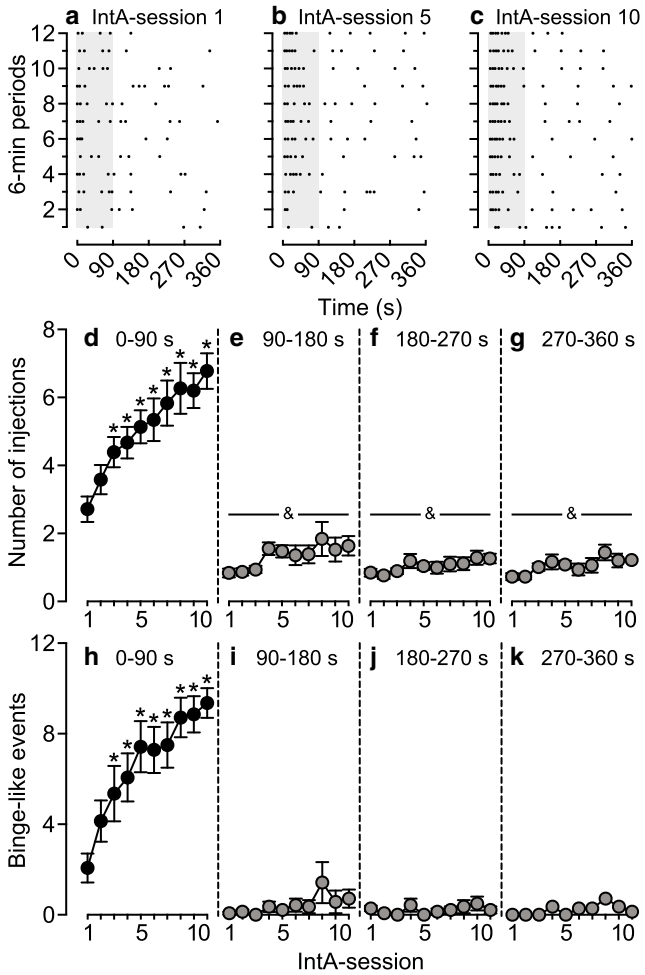

Fig. 3 Rats self-administering cocaine during intermittent-access sessions (IntA) develop a binge-like pattern of drug intake. The pattern of cocaine intake is shown for a representative rat during the 1st (**a**), 5th (**b**), and 10th (**c**) IntA-session. Each point represents one self-administered injection. The *Y*-axis shows self-administered infusions during each 6-min cocaine period. The *X*-axis shows time in 90-s (s) blocks. On sessions 5 and 10, the rat took most of its injections at the beginning of each 6-min drug period (the first 90 s; shaded in gray). **d**–**g** The number of cocaine injections taken during each 6-min drug period, broken down into 90-s bins. **d** The rats took most of their cocaine injections in the first 90-s bin of each 6-min drug period and this "loading" effect sensitized over sessions. **h**–**k** Binge-like events (≥ 5 injections/90 s) during each 6-min drug period, broken down into 90-s bins. **h** Binge-like events were observed only in the first 90 s of the 6-min cocaine periods, and binge-like behavior sensitized over IntA-sessions. $^{*}p < 0.05$, vs. 1st IntA-session. $^{\&}p < 0.05$, main effect of session. Data are mean ± s.e.m. $n = 14$

Experiment 2

Experiment 1 showed that high and escalating levels of cocaine intake under IntA conditions do not significantly predict later incentive motivation for the drug. At this point, the data remain correlational. Thus, in Experiment 2, we determined whether taking high and escalating amounts of cocaine under IntA conditions is sufficient to produce increased incentive motivation for the drug. If this is true, then IntA-rats that escalate their intake should achieve higher breakpoints for cocaine than IntA-rats precluded from escalating their intake. We tested this prediction by comparing two groups of IntA-rats. During each IntA-session, one group had unlimited access to cocaine to permit escalation (IntA-unLim) while the second group was limited to four injections/6-min drug period (IntA-Lim) to preclude escalation (Fig. 1b). This cocaine injection limit was based on Experiment 1, which showed that rats take 4–5 injections/6-min cocaine period on the 1st IntA-session. Figure 5a shows intake patterns and estimated brain concentrations of cocaine in representative animals. IntA-Lim rats take less cocaine than IntA-unLim rats. Thus, spikes in estimated brain cocaine levels are of smaller amplitude in IntA-Lim rats (Fig. 5a; black line). Note that self-administration sessions can be shorter in IntA-Lim rats because each cocaine period within the session ends as soon as four injections are taken, or after 6 min.

Limiting the number of cocaine injections available during each IntA-session precludes escalation of cocaine intake

IntA-Lim rats consumed significantly less cocaine over sessions than IntA-unLim rats (main effect of group, $F_{1,\ 18} = 13.75$, $p = 0.002$; Fig. 5b), and only IntA-unLim rats escalated their intake over time (main effect of session, $F_{9,\ 162} = 8.05$, $p < 0.0001$; group × session interaction effect, $F_{9,\ 162} = 4.64$, $p < 0.0001$; Fig. 5b). From the 4th session on, IntA-unLim rats took more cocaine than on their 1st session (all p values < 0.01; Fig. 5b). Accordingly, cumulative cocaine intake was greatest in IntA-unLim rats ($t_{18} = 3.71$, $p = 0.002$; Fig. 5c). The two groups showed a similar increase in the rate of cocaine intake over sessions, as indicated by a decrease in the inter-injection interval (main effect of session, $F_{9,\ 162} = 19.29$, $p < 0.0001$; no other comparisons were statistically significant; Fig. 5d). Note that in the IntA-unLim rats, the inter-injection interval was calculated only for the first four injections taken in each 6-min drug period of the IntA-session. This permits direct comparison with the IntA-Lim rats, which were limited to four injections/6-min drug period.

Under IntA conditions, high and escalating levels of cocaine intake do not increase incentive motivation for the drug in the future

Following the 10th IntA-session, breakpoints maintained by cocaine were measured under a PR schedule of reinforcement. Across groups, both breakpoint (main effect of dose, $F_{2,\ 36} = 11.59$, $p = 0.0001$; Fig. 5e) and session duration (main effect of dose, $F_{2,\ 36} = 21.15$, $p < 0.0001$; Fig. 5f–h insets) increased as a function of cocaine dose. There were no group differences in either measure. Cumulative breakpoints during the PR-sessions were also similar between the IntA-unLim and IntA-Lim rats (Fig. 5f–h). In summary, the IntA-unLim rats escalated their intake and took twice the amount of

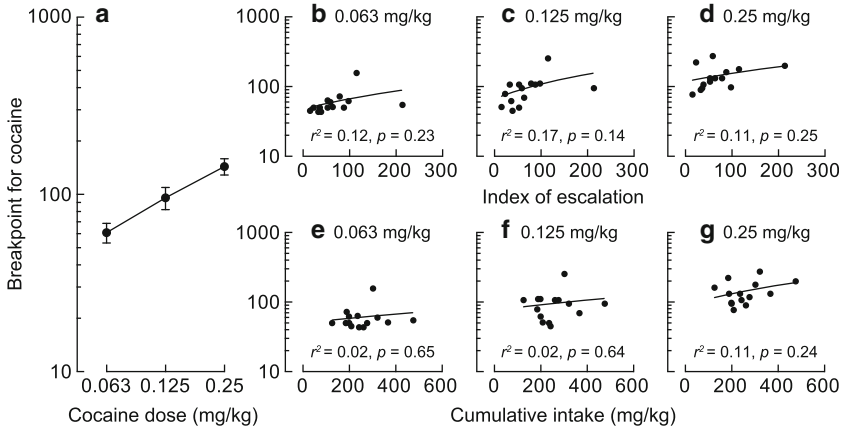

Fig. 4 Breakpoint for cocaine under a progressive ratio schedule of reinforcement increases with dose (**a**) and does not significantly correlate with the degree of prior escalation of drug intake (**b**–**d**) or with prior cumulative cocaine intake (**e**–**g**). **a** shows breakpoints maintained by cocaine on a log scale, as a function of cocaine dose. **b**–**d** show correlations between breakpoints for different doses of cocaine and the extent of prior escalation of cocaine intake. **e**–**g** show correlations between breakpoints for different doses of cocaine and previous cumulative cocaine intake. Data are mean ± s.e.m. $n = 14$

cocaine than IntA-Lim rats, but this was not sufficient to produce differences in the later incentive motivation for the drug.

Experiment 3

If high and escalating amounts of cocaine intake are necessary to produce increased incentive motivation for the drug, then LgA-rats that escalate their intake should achieve higher breakpoints for cocaine than IntA-rats precluded from escalating their intake. Thus, one group was given LgA-sessions during which cocaine access was not limited (LgA-rats). A second group was given IntA-sessions and was limited to two injections/6-min drug period (IntA-Lim rats) so as to preclude escalation (Fig. 1c). In contrast to Experiment 2, here, IntA-Lim rats were limited to two instead of four injections/drug period [as used previously in Allain et al. (2017)]. This maximizes differences in intake between IntA-Lim rats and LgA-rats. Figure 6a shows patterns of cocaine intake and estimated brain concentrations of drug in representative rats. IntA-rats (black line) show spikes and troughs in estimated brain cocaine concentrations, while LgA-rats show high and sustained estimated brain concentrations [also see (Zimmer et al. 2012)].

IntA-Lim produces low and stable levels of cocaine intake while LgA promotes high and escalating levels of cocaine intake over sessions

Figure 6b shows cocaine intake over sessions. IntA-Lim rats showed stable intake over time (Fig. 6b). However, LgA-rats escalated their intake over time (main effect of session, $F_{9, 117} = 5.11$, $p < 0.0001$; group × session interaction, $F_{9, 117} = 4.6$, $p < 0.0001$; Fig. 6b). From the 3rd session on, they took more cocaine than on the 1st session (all p values < 0.01). LgA-rats also took 6–9 times more cocaine/session than IntA-Lim rats (main effect of group, $F_{1, 13} = 280$, $p < 0.0001$; Fig. 6b). Average cumulative cocaine intake over the 10 self-administration sessions was eightfold higher in LgA compared to IntA-Lim rats ($t_{13} = 16.73$, $p < 0.0001$; Fig. 6c). Both groups pressed more on the active than on the inactive lever (main effect of lever type; LgA-rats, $F_{1, 7} = 360.6$; IntA-Lim rats, $F_{1, 6} = 125.7$; all p values < 0.0001; Fig. 6d), and active lever presses increased over sessions in the LgA-rats (main effect of session, $F_{9, 63} = 5.63$; lever type × session interaction, $F_{9, 63} = 5.36$; all p values < 0.0001; Fig. 6d).

IntA without escalation of cocaine intake produces greater incentive motivation for cocaine than LgA with escalation

Following the 10th self-administration session, breakpoints maintained by cocaine were measured under a PR schedule of reinforcement. Across groups, breakpoints for cocaine increased as a function of dose (main effect of dose, $F_{2, 26} = 15.04$, $p < 0.0001$; Fig. 6e). However, the dose-response curve was shifted upwards in the IntA-Lim rats (group × cocaine dose interaction effect, $F_{2, 26} = 3.44$, $p < 0.05$; main effect of group, $F_{1, 13} = 3.4$, $p = 0.09$; Bonferroni's test at the 0.25-mg/kg dose, $p = 0.02$; no other comparisons were statistically significant; Fig. 6e). During PR-sessions, group differences in breakpoint for cocaine appeared early and were persistent (Fig. 6f–h). PR-sessions also lasted longest in IntA-Lim rats (main effect of group, $F_{1, 13} = 31.31$, $p < 0.0001$; Fig. 6f–h insets). This indicates that when obtaining cocaine required increasing amounts of physical effort, IntA-Lim rats persevered longer in the self-administration task compared to LgA-rats. In summary, LgA-rats escalated their intake over time and took significantly more cocaine than IntA-Lim rats, but IntA-Lim rats developed greater incentive motivation for the drug.

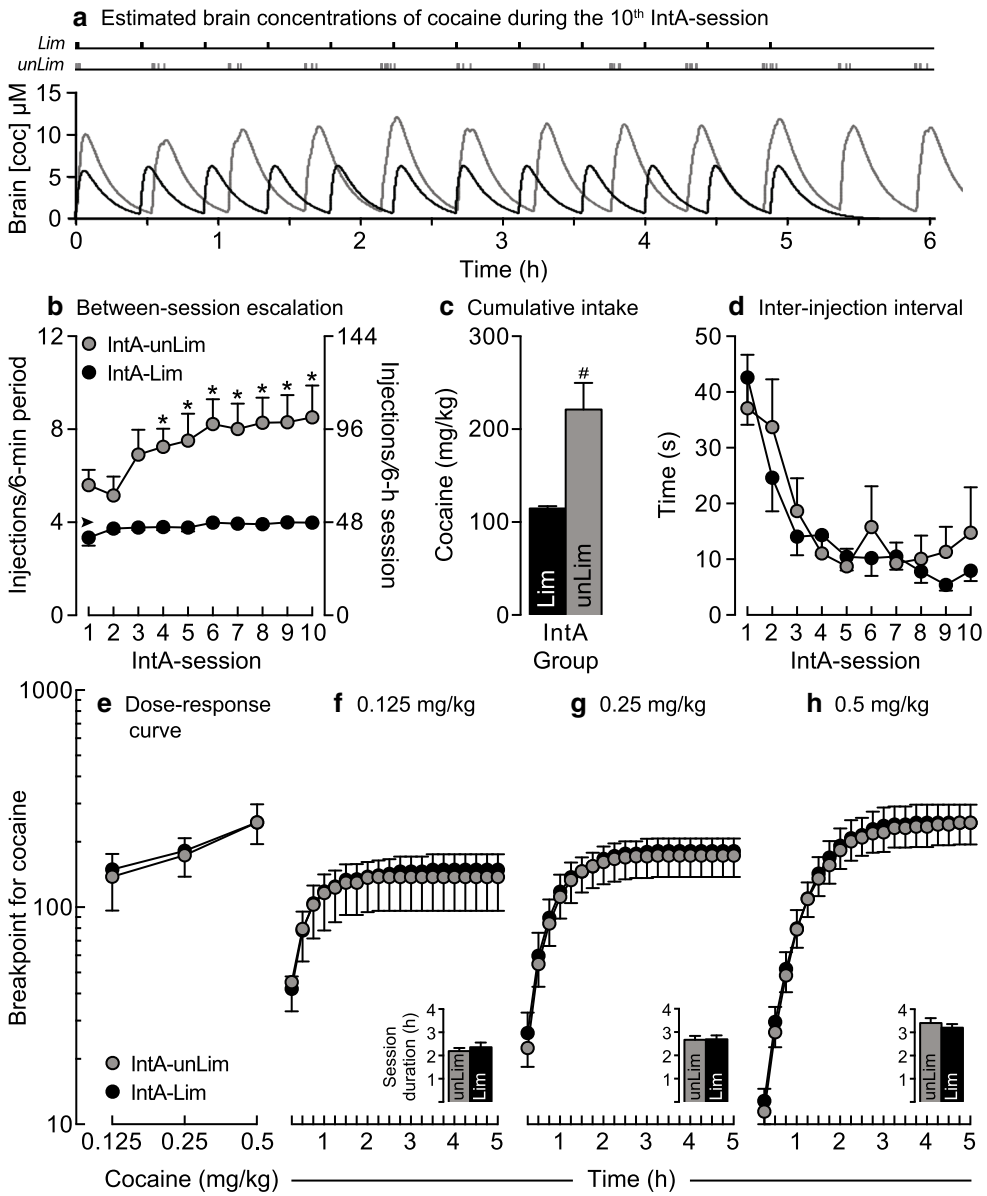

Fig. 5 Under intermittent-access conditions (IntA), high and escalating levels of cocaine intake do not promote increased motivation to obtain the drug compared to low and stable levels of intake. **a** Patterns of cocaine intake (top two lines) and estimated brain cocaine concentrations as a function of time during the 10th self-administration session in representative animals from each group. In **b**, the left *Y*-axis shows the number of injections taken/6-min cocaine period and the right *Y*-axis shows the total number of injections taken/session. The arrow indicates that IntA-Lim rats were limited to four injections/6-min drug period. IntA-unLim rats had unlimited access to cocaine during each 6-min drug period. **c** Cumulative cocaine intake was greatest in unLim rats. **d** The inter-injection interval [in seconds (s)] was similar between groups and it decreased over sessions. $^{*}p < 0.05$, vs. 1st IntA-session in IntA-unLim rats. $^{\#}p < 0.05$, vs. IntA-Lim group. **e** IntA-Lim and IntA-unLim rats showed similar breakpoints for cocaine under a progressive ratio schedule of reinforcement. The panel shows breakpoint values on a log scale, as a function of cocaine dose. **f**–**h** Cumulative breakpoint for cocaine during progressive ratio tests as a function of time. The insets in **f**–**h** show the duration of progressive ratio sessions in each group. Data are mean ± s.e.m. $n = 10$/group

Discussion

We show that high and escalating levels of cocaine intake are neither sufficient nor necessary to produce an increase in incentive motivation for the drug [see also Zimmer et al. (2012)]. First, we found that rats that self-administer cocaine under IntA significantly escalate their drug intake both within and between sessions. However, neither the extent of escalation nor the cumulative amount of cocaine taken significantly predicted incentive motivation for the drug (Fig. 4). We then compared two groups of IntA animals. In one group, we limited the number of cocaine injections available such that intake was low and escalation was precluded (IntA-Lim). In the second group, the number of injections was not limited and these

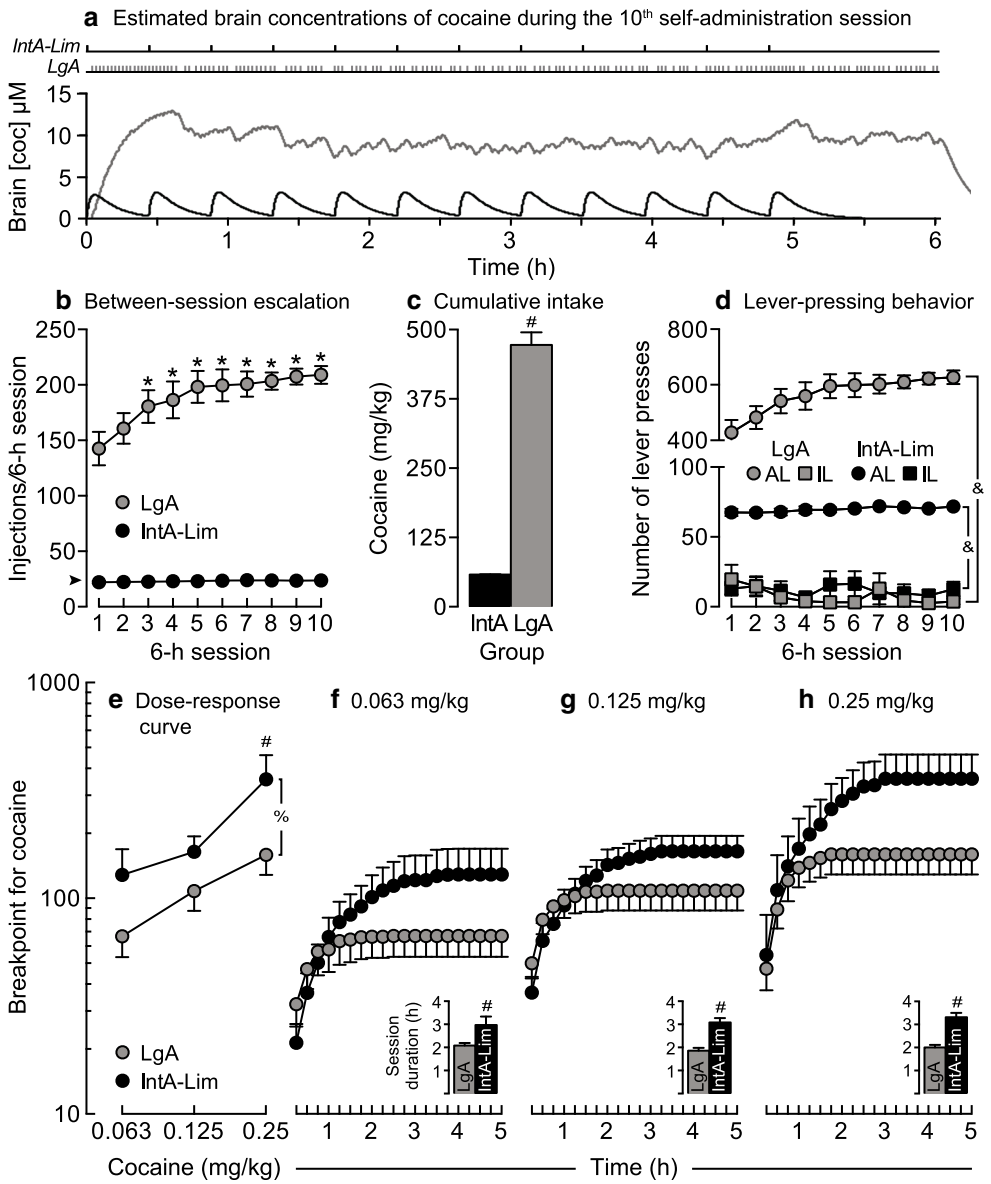

Fig. 6 Compared to LgA-rats, IntA-Lim self-administer significantly less cocaine but develop greater incentive motivation to take the drug. **a** Patterns of intake (top two lines) and estimated brain cocaine concentrations as a function of time during the 10th session in representative animals of each group. **b** LgA-rats took significantly more cocaine injections during each session than IntA-Lim rats and also escalated their intake. The arrow indicates that IntA-Lim rats were limited to two injections/6-min drug period. LgA-rats had unlimited drug access save for an 85-s timeout period following each injection. **c** Cumulative cocaine intake was greatest in LgA-rats. **d** Both groups pressed significantly more on the active lever (AL, circle symbols) versus the inactive lever (IL, square symbols) during each 6-h session. **e** Compared to LgA-rats, IntA-Lim rats reached higher breakpoints for cocaine under a progressive ratio schedule of drug reinforcement. The panel shows breakpoint values on a log scale as a function of cocaine dose. **f**–**h** Cumulative breakpoint for cocaine during progressive ratio tests as a function of time. The insets in **f**–**h** show that progressive ratio sessions were longest in IntA-Lim rats, at all doses tested. This indicates that they persevered longer in the progressive ratio task compared to LgA-rats. $^{*}p < 0.05$, vs. 1st 6-h session in LgA-rats. $^{\#}p < 0.05$, IntA-Lim rats vs. LgA-rats. $^{\&}p < 0.0001$, main effect of lever type. $^{\%}p < 0.05$, Group × Dose interaction effect. Data are mean ± s.e.m. n = 7–8/group

rats took high and escalating amounts of cocaine (IntA-unLim). However, both groups later showed similar levels of incentive motivation for cocaine (Fig. 5). Next, we compared IntA-Lim rats to LgA-rats. LgA-rats had virtually continuous cocaine access during each session, and only LgA-rats escalated their intake, taking ~ eightfold more cocaine than IntA-Lim rats. However, the IntA-Lim rats developed greater incentive motivation for cocaine (Fig. 6). These findings challenge the assumption that high and escalating levels of cocaine intake are sufficient and necessary to increase the motivation to take the drug (Ahmed and Koob 1998; Hao et al. 2010; Paterson and Markou 2003), at least under IntA-conditions. In agreement with Zimmer et al. (2012), the results also show that compared to continuously high and escalating brain levels

of cocaine, intermittently spiking levels more effectively increase incentive motivation to take drug. We compared incentive motivation for cocaine in IntA- and LgA-rats at a single time point, 1–4 days after the last self-administration session. Incentive motivation for cocaine can change over the withdrawal period (Calipari et al. 2015) and we do not know how IntA- and LgA-rats would compare after different withdrawal times. Nonetheless, our findings suggest that beyond how much drug is taken, the temporal pattern of drug use is decisive in producing change in drug use over time (Allain et al. 2015). In further support of this idea, Deroche et al. (1999) have shown that allowing rats to self-administer cocaine during sessions that include drug-free periods between drug available periods—similar to the procedure used here—produces robust sensitization to the incentive motivational effects of the drug.

IntA and LgA both produced escalation of cocaine intake, but the escalation effect was qualitatively different. IntA-rats escalated their intake *between*, and also *within*, sessions, taking more drug during each consecutive cocaine period (Fig. 2b). This was particularly marked at the beginning of each 6-min drug period, when brain cocaine concentrations are low (Fig. 3). IntA-rats also showed multiple binge-like episodes of cocaine use (≥ 5 injections/90 s) within each self-administration session, and this effect sensitized over time (Fig. 3). Thus, the distinct spiking pattern in brain cocaine concentrations produced by the IntA procedure promotes intermittent episodes of high-frequency drug intake. Such episodes are thought to facilitate the emergence of addiction-like symptoms (Belin et al. 2009; Martin-Garcia et al. 2014).

LgA versus IntA experience could produce escalation via different psychological and neurobiological mechanisms. Kawa et al. (2016) hypothesized that escalation under LgA involves tolerance to the subjective pleasurable effects of the drug [also see (Ahmed 2012; Calipari et al. 2014a; Edwards and Koob 2013)], while escalation under IntA involves sensitization to the incentive motivational effects of drug. Compared to LgA, IntA more effectively produces sensitization of incentive motivation for cocaine [(Zimmer et al. 2012) and present data]. IntA to cocaine also evokes robust psychomotor sensitization, and the degree of psychomotor sensitization predicts subsequent incentive motivation for cocaine in rats with IntA-experience (Allain et al. 2017). In contrast, psychomotor sensitization is not generally observed following LgA-experience (Ahmed and Cador 2006; Ben-Shahar et al. 2004; Knackstedt and Kalivas 2007). This could depend on when animals are tested, because when LgA-rats are tested after extended abstinence, they can indeed show psychomotor sensitization (Ferrario et al. 2005). LgA- versus IntA-experience also produces opposite effects on dopamine. IntA-rats develop sensitization to cocaine-, methylphenidate-, and methamphetamine-induced inhibition of the dopamine transporter in the nucleus accumbens, while LgA-rats develop tolerance to cocaine's effects at the transporter, at least when tested on the day following the last cocaine self-administration session (Calipari et al. 2014b; Calipari et al. 2013). This agrees with evidence that injecting rats intermittently with cocaine evokes sensitization of the drug's effects on dopamine reuptake, while exposing rats to cocaine continuously promotes tolerance (Izenwasser and Cox 1990, 1992; Post 1980).

Our findings concord with others showing that addiction-relevant symptoms can develop without escalation of intake. For instance, cocaine self-administration under either IntA or LgA conditions without escalation can still increase incentive motivation for the drug (Bouayad-Gervais et al. 2014; Kippin et al. 2006; Minogianis et al. 2013; Zimmer et al. 2012). Similarly, short-access sessions (ShA; continuous drug access for 1–3 h/session) often produce stable levels of drug intake, but they can evoke both psychomotor sensitization and sensitized drug-induced dopamine release, particularly after an abstinence period (Hooks et al. 1994). Even in animals given prolonged access to cocaine (> month), the amount of drug taken does not predict the later susceptibility to addiction-relevant behaviors (Belin et al. 2009; Deroche-Gamonet et al. 2004). In fact, taking too much cocaine can prevent sensitization to the incentive motivational effects of the drug (Li et al. 1994; Morgan et al. 2006; Morgan et al. 2005; Roberts et al. 2002), perhaps by evoking tolerance-related neuroadaptations (Calipari et al. 2013). As such, while taking high and escalating levels of drug is a diagnostic criterion for drug addiction (APA 2013), escalation might be a consequence, rather than a cause in the transition to addiction. If this is true, it has strong implications for modeling in animals the changes in brain and psychological function that promote the transition from casual drug use to addiction.

Conclusions

In summary, using an IntA drug self-administration procedure, the present results show that high and escalating levels of cocaine intake are neither sufficient nor necessary to produce increased incentive motivation for the drug. This questions the idea that "The continued use of intake escalation models will (…) reveal the most suitable strategies for therapeutic intervention" (Edwards and Koob 2013). IntA cocaine self-administration experience most effectively produces addiction-relevant behaviors. This includes binge-like, high-frequency drug use, robust psychomotor sensitization, increased incentive motivation for drug [(Allain et al. 2017; Kawa et al. 2016; Zimmer et al. 2011; Zimmer et al. 2012) and present data], a progressive decrease in the elasticity of the cocaine demand curve, a progressive increase in the willingness to work for cocaine despite an adverse consequence, and greater cue-induced reinstatement of cocaine-seeking behavior than usually seen in LgA-rats (Kawa et al. 2016). The IntA

procedure might also more closely model how human addicts take cocaine (Beveridge et al. 2012). Yet, IntA leads to much less drug intake than LgA. In addition, IntA versus LgA-experience can produce different—even opposite—effects on dopamine neurotransmission (Calipari et al. 2013). Together, this literature and the present findings challenge long-held beliefs about what constitutes a good animal model of drug addiction. Given this, there must be further investigations to determine which model is more useful in producing the neuroadaptations that underlie the transition to addiction (Kawa et al. 2016).

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Author contributions F.A performed research and analyzed the data. F.A and A.N.S designed the research and wrote the paper. F.A and K.B.G wrote and tested the computer code needed to apply the IntA drug self-administration procedure to the operant conditioning cages in the laboratory.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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