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Revisiting long-access versus short-access cocaine self-administration in rats: intermittent intake promotes addiction symptoms independent of session length

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ABSTRACT

In rats, continuous cocaine access during long self-administration sessions (6 versus 1–2 hours) promotes the development of behavioral symptoms of addiction. This has led to the assumption that taking large amounts of drug during extended daily bouts is necessary to develop an addiction phenotype. Recent work shows that within-session intermittent access (IntA) to cocaine produces much less drug intake than continuous-access procedures (i.e. long-access sessions) but evokes addiction symptoms more effectively. IntA-sessions are also long, typically lasting 6 hours. It is not known whether IntA-sessions must be extended to promote addiction-relevant changes in drug use over time. Here, we determined the influence of IntA-session length on patterns of cocaine use relevant to addiction. Two groups of male Wistar rats self-administered cocaine (0.25 mg/kg/injection, injected over 5 seconds) during 18 daily IntA-sessions. One group had long 6-hour sessions (Long-IntA), the other group had shorter, 2-hour sessions (Short-IntA). Only Long-IntA rats escalated their cocaine intake over sessions, but both groups developed a burst-like pattern of drug use over time and similar levels of psychomotor sensitization. The two groups also showed robust and similar levels of both responding for cocaine under a progressive ratio schedule of reinforcement and cocaine-induced reinstatement of extinguished drug-seeking behavior. In summary, long IntA-sessions lead to greater cocaine intake than shorter IntA- sessions, but the two conditions are equally effective in evoking the patterns of drug-taking and drug-seeking that define addiction. This suggests that chronic intermittent cocaine use, even during short daily bouts, is sufficient to promote addiction symptoms.

Keywords burst-like intake, drug-induced reinstatement, intermittent access to cocaine, progressive ratio, psychomotor sensitization, session duration.

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INTRODUCTION

Drug addiction is a gradual process whereby vulnerable users progress from recreational to pathological drug use (Gawin 1991). A challenge in addiction research is to model this transition in animals, such that changes in drug self-administration behavior are seen over time (Roberts, Morgan, & Liu 2007). Currently, a commonly used model involves giving rats continuous access to drug during long, 6-hour self-administration sessions [Long-access or LgA-sessions; (Ahmed & Koob 1998)]. Relative to shorter daily sessions (typically 1–2 hours), LgA can promote robust escalation of cocaine intake over time (Ahmed & Koob 1998, 1999; Mantsch *et al.* 2004; Hao, Martin-Fardon, & Weiss 2010; Bouayad-Gervais *et al.* 2014; Mandt *et al.* 2015), increased motivation to obtain cocaine (Paterson & Markou 2003; Hao *et al.* 2010) and greater susceptibility to cocaine-induced relapse after abstinence (Mantsch *et al.* 2004; Knackstedt & Kalivas 2007). Such findings have led to the proposal that taking large amounts of drug continuously during extended sessions is necessary to develop behavioral symptoms of addiction (Ahmed & Koob 1998; Ahmed 2012; Edwards & Koob 2013).

Recent work has begun to challenge this belief. Zimmer, Dobrin, & Roberts (2011) developed an

intermittent-access (IntA) drug self-administration procedure in animals. Similar to LgA-sessions, IntA-sessions are also extended, lasting 4–6 hours. However, in contrast to LgA where access to drug is continuous within sessions, IntA gives animals access to drug during 5-minute periods intercalated with 25-minute no-drug periods. This achieves peaks and troughs in brain cocaine concentrations during each daily session, in contrast to the continuously high concentrations achieved during LgA-sessions (Zimmer, Oleson, & Roberts 2012). This distinction is important, as human cocaine addicts also appear to take cocaine intermittently within a bout of consumption [(Beveridge *et al.* 2012); see Allain *et al.* 2015 for review], and this would presumably achieve a spiking pattern in brain cocaine concentrations (Zimmer *et al.* 2011). In further support of this idea, cocaine users can also experience several episodes of euphoria within a bout of intake (Gawin 1991)—suggesting that cocaine levels in blood/brain rise and fall within such bouts. However, systematic studies on the temporal pattern with which human addicts take cocaine during a bout of intoxication are lacking. Such work is needed to inform drug self-administration procedures in laboratory animals. It is also important to note that while cocaine addicts might voluntarily consume the drug intermittently within a bout of intoxication, the IntA procedure imposes an intermittent pattern of cocaine self-administration on rats.

These issues notwithstanding, comparing IntA-rats to LgA-rats has produced surprising findings. Just like LgA-rats, IntA-rats can also significantly escalate their drug intake over time (Kawa, Bentzley, & Robinson 2016; Pitchers *et al.* 2017; Allain, Bouayad-Gervais, & Samaha 2018). However, although IntA-rats take much less cocaine than LgA-rats, IntA-rats develop greater incentive motivation for the drug, as measured either by responding for the drug under a progressive ratio (PR) schedule of reinforcement or using behavioral economics procedures (Zimmer *et al.* 2012; Allain *et al.* 2018). IntA-rats also show a progressive decrease in the elasticity of the cocaine demand curve, a gradual increase in the willingness to work for cocaine despite an adverse consequence and stronger cue-induced reinstatement of cocaine-seeking behavior than generally seen in LgA-rats (Kawa *et al.* 2016). In addition, IntA-rats develop a burst-like pattern of cocaine use, as indicated by multiple episodes of high-frequency intake during each session, where rats load up on cocaine each time the drug becomes available again (Allain *et al.* 2018). Such burst-like cocaine use is thought to facilitate the development of addiction (Belin *et al.* 2009; Martin-Garcia *et al.* 2014). Because drug access is continuous during an LgA-session, brain cocaine concentrations would remain high (Zimmer *et al.* 2012; Allain *et al.* 2018), and this would likely not evoke repeated episodes of burst-like drug use within the session. However, at this stage, this prediction remains speculative, as the within-session pattern of cocaine intake in LgA-rats has not been studied systematically. Finally, IntA-rats are sensitized to cocaine-, methylphenidate- and methamphetamine-induced blockade of the dopamine transporter in the nucleus accumbens, while LgA-rats show tolerance to cocaine-induced inhibition of the transporter (Calipari *et al.* 2013; Calipari *et al.* 2014). Thus, IntA to cocaine more effectively induces the changes in brain and behavior that are relevant to addiction.

Similar to the LgA procedure, IntA also involves long daily sessions (4–6 hours), but it is not known whether this is necessary to produce addiction-relevant patterns of drug use. To our knowledge, no one has assessed the influence of session length using the IntA procedure. However, there is evidence that even very brief exposure to IntA cocaine self-administration (as little as three 6-hour sessions) can produce sensitization to both the incentive motivational effects of cocaine as measured using behavioral economics indices, and the drug's effects at the dopamine transporter (Calipari *et al.* 2015). Here, we sought to determine the influence of IntA-session length on outcome. This is important because it has implications for modeling in animals the changes in brain and psychological function that underlie the progression from recreational drug use to drug addiction. We compared two groups of rats allowed to self-administer cocaine during IntA-sessions. One group had 6-hour IntA-sessions (Long-IntA) while the second group had 2-hour IntA-sessions (Short-IntA). We compared changes in the response to cocaine following chronic self-administration of the drug. We assessed escalation of intake, the emergence of a burst-like pattern of drug use, the development of psychomotor sensitization, the motivation to take cocaine under a PR schedule of drug reinforcement and the vulnerability to cocaine-induced reinstatement of extinguished drug-seeking after abstinence. If session length is a critical factor in the development of an addiction phenotype, then Long-IntA rats should preferentially show these behavioral changes compared to Short-IntA rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (n = 27, 225–250 g; Charles River Laboratories, St Constant, QC) were housed individually under a reverse 12 hours–12 hours dark–light cycle (Lights off at 8:30 AM) in a temperature-controlled and humidity-controlled room. After 4–5 days of habituation to the animal colony, rats were implanted with a catheter into the jugular vein, as described previously (Samaha, Minogianis, & Nachar 2011). Water was

available ad libitum and food was restricted to 25 g/day. The animal ethics committee of the Université de Montréal approved all procedures involving rats, and these followed the guidelines of the Canadian Council on Animal Care.

Acquisition of food and cocaine self-administration

Figure 1 illustrates the experimental design. All behavioral testing took place during the dark phase of the rats' circadian cycle. After 3–4 days of recovery from surgery, rats were placed in standard operant cages (Med Associates, St Albans, VT) and trained to press a lever to self-administer 45-mg, banana-flavored, grain-based food pellets (VWR, Town of Mount-Royal, QC) in daily 1-hour sessions, under a fixed ratio 3 schedule of reinforcement (FR3, three lever presses give one food pellet). This was performed to hasten the acquisition of the lever-pressing response, and thus decrease the time needed to later learn to self-administer cocaine. The house-light was illuminated at the beginning of each test session and was turned off when the session ended. Rats had access to an active and an inactive lever. Pressing the active lever was reinforced with a food pellet. Pressing the inactive lever had no programmed consequences. When rats showed reliable lever-pressing for food (>25 pellets/session, on two consecutive sessions), food pellets were substituted with intravenous (i.v.) injections of 0.25-mg/kg cocaine, delivered over 5 seconds. Each cocaine injection was followed by a 20-second timeout period. During each drug injection and timeout period, both levers were retracted and the cue light above the active lever was illuminated. Rats that self-administered at least six cocaine injections at regular intervals on two consecutive days and pressed ≥2 times more on the active versus inactive lever were assigned to one of two groups. All rats took an average of 4 days (3–7 days) to meet these criteria. Group assignment was made such that number of self-administered cocaine injections and number of lever presses were similar in both groups. Both groups were now given 18 daily IntA-sessions. One group had 2-hour sessions (Short-IntA rats) and the other group had 6-hour sessions (Long-IntA rats).

Catheter patency was checked after the last IntA-session and after the last PR-session by giving rats i.v. Propofol (1 mg per 0.1 ml, CDMV, St-Hyacinthe, QC). All rats became ataxic after this injection. Three rats were excluded during cocaine training because they did not meet acquisition criteria after 1 week. Two Short-IntA rats and one Long-IntA rat were excluded from the experiment because they did not reliably self-administer cocaine during IntA-sessions (taking on average 0–1 injection/5-minute drug period throughout the 18 IntA-sessions).

Intermittent access to cocaine

During IntA-sessions (Zimmer *et al.* 2011; Zimmer *et al.* 2012; Allain *et al.* 2017; Allain *et al.* 2018), Short-IntA and Long-IntA rats could self-administer cocaine (0.25-mg/kg/inj, injected over 5 seconds) during 5-minute periods, followed by 25-minute no-cocaine periods, during which both levers were retracted and no cocaine was available. During each 5-minute drug period, cocaine was available under FR3, and there was no timeout period after each drug injection. Our prior work shows that under these conditions, Long-IntA rats develop a burst-like pattern of cocaine intake (Allain *et al.* 2018). Short-IntA rats had *four* 5-minute cocaine periods/session while Long-IntA rats had *twelve* 5-minute cocaine periods/session (Fig. 3a). During each IntA-session, we also measured locomotor behavior using four infrared photocells, aligned horizontally in each operant cage. Locomotor activity was computed as photocell beam breaks/minute.

We estimated brain cocaine concentrations (C; in μM) as a function of time 't' during the 1st and 18th IntA-sessions in a representative rat from each group using the following formula:

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

This was performed using a mathematical 2-compartment open model for rats treated chronically with intravenous cocaine injections (Pan, Menacherry,

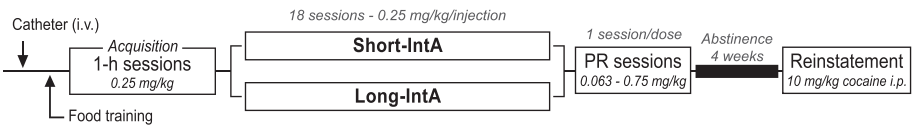

Figure 1 The sequence of experimental events. Following the acquisition of operant responding first for food pellets and then for cocaine (0.25 mg/kg/inj), two groups of rats were allowed to self-administer cocaine (0.25 mg/kg/inj) during 18 intermittent-access sessions (IntA). Sessions lasted 2 hours in one group (Short-IntA) and 6 hours in the other group (Long-IntA). During each IntA-session, cocaine was available for 5-minute periods, separated by 25-minute no-cocaine periods. Next, breakpoints for cocaine were assessed under a progressive ratio (PR) schedule of reinforcement. Finally, after 1 month of abstinence from cocaine, we assessed cocaine- (10 mg/kg, i.p.) induced reinstatement of extinguished cocaine-seeking behavior

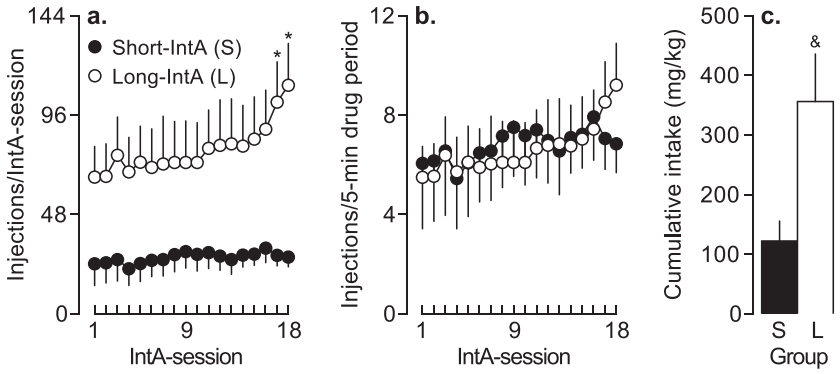

Figure 2 Extended IntA-sessions (6 hours) promote high and escalating levels of cocaine intake while shorter IntA-sessions (2 hours) produce low and stable levels of intake. (a) Average number of cocaine injections per session and (b) average number of cocaine injections per 5-minute cocaine period. Note that each 2-hour session had *four* 5-minute cocaine periods, while each 6-hour session had *twelve* 5-minute cocaine periods. Panel (c) shows cumulative cocaine intake over the 18 IntA-sessions in the two groups. Long-IntA (L) rats escalated their cocaine intake and self-administered threefold more cocaine than Short-IntA (S) rats. $^{*}P < 0.05$, versus first Long-IntA session. $^{\&}P < 0.05$, Long-IntA versus Short-IntA rats. Data are mean ± SEM. n = 10–11/group

& Justice Jr 1991). 'd' represents the self-administered cocaine dose per injection (0.25 mg · kg^{-1}). 'A' (9.63 μM · mg^{-1} · kg) integrates the rate constant 'k' for transfer of cocaine from blood to brain, the apparent volume 'v' of brain distribution and two constants 'α' (0.6419 min^{-1}) and 'β' (0.0971 min^{-1}) that account for removal of cocaine from the system via redistribution or elimination. Finally, 't' is the time in minutes since the last cocaine injection. Dr. David C. S. Roberts generously provided the Python script used to model brain cocaine concentrations.

Progressive ratio and cocaine-induced reinstatement of extinguished drug-seeking

After 18 IntA-sessions, rats were kept in their home cages for 4 days. The rats were then tested under a progressive ratio (PR) schedule of drug reinforcement to assess incentive motivation for cocaine (0.063, 0.125, 0.25, and 0.75 mg/kg/inj; 1 day/dose; tested in counterbalanced order save for the highest dose which was tested last). To remain constant with previous work (Allain *et al.* 2017; Allain *et al.* 2018), we used a standard PR procedure, where both levers are present in the cage throughout the session, except during each 5-second injection (Richardson & Roberts 1996). Under PR, the number of active lever presses required to obtain each successive injection increased exponentially according to the following formula:

$$\left[5 \times e^{(number\ of\ injection \times 0.2)} - 5\right] \text{(Richardson \& Roberts 1996)}$$

PR-sessions lasted 5 hours or stopped after 1 hour of no drug intake. The last ratio reached is the breakpoint and it is used as an index of the motivation for cocaine. After the last PR-session, rats were kept in their home cages for 4 weeks and were handled regularly. Then, cocaine-induced reinstatement of extinguished cocaine-seeking behavior was assessed. Rats received a 6-hour extinction session immediately followed by a 2-hour cocaine-induced reinstatement session. During these sessions, both levers were present in the test cage but lever-pressing produced no cocaine or cocaine cues. Immediately before the 2-hour reinstatement session, rats received 10 mg/kg cocaine i.p.

Data analysis

Group differences in cocaine intake over IntA-sessions was analyzed using a 2-way repeated-measures ANOVA (Group × IntA-session, the latter as a within-subjects variable). Cumulative cocaine intake was compared between groups with an unpaired t-test. The number of episodes of burst-like intake (≥3 self-administered injections/minute) during each minute of the 5-minute cocaine periods was further analyzed using 2-way repeated-measures ANOVA (1-minute bin × IntA-session, both as within-subjects variables) and 1-way repeated-measures ANOVA (changes within each 1-minute bin, across IntA-sessions). Locomotor activity was compared between IntA-sessions 1 and 18 using 2-way repeated-measures ANOVA (Session × Time, both as within-subjects variables; Group × Time, the latter as a within-subjects variable). Breakpoints for cocaine achieved under PR were analyzed using 2-way repeated-measures ANOVA (Group × Dose of cocaine, the latter as a within-subjects variable). Three-way repeated-measures ANOVAs were used to both analyze lever-pressing behavior during extinction (Group × Lever type × Hour, the latter two as within-subjects variables) and to compare lever-pressing

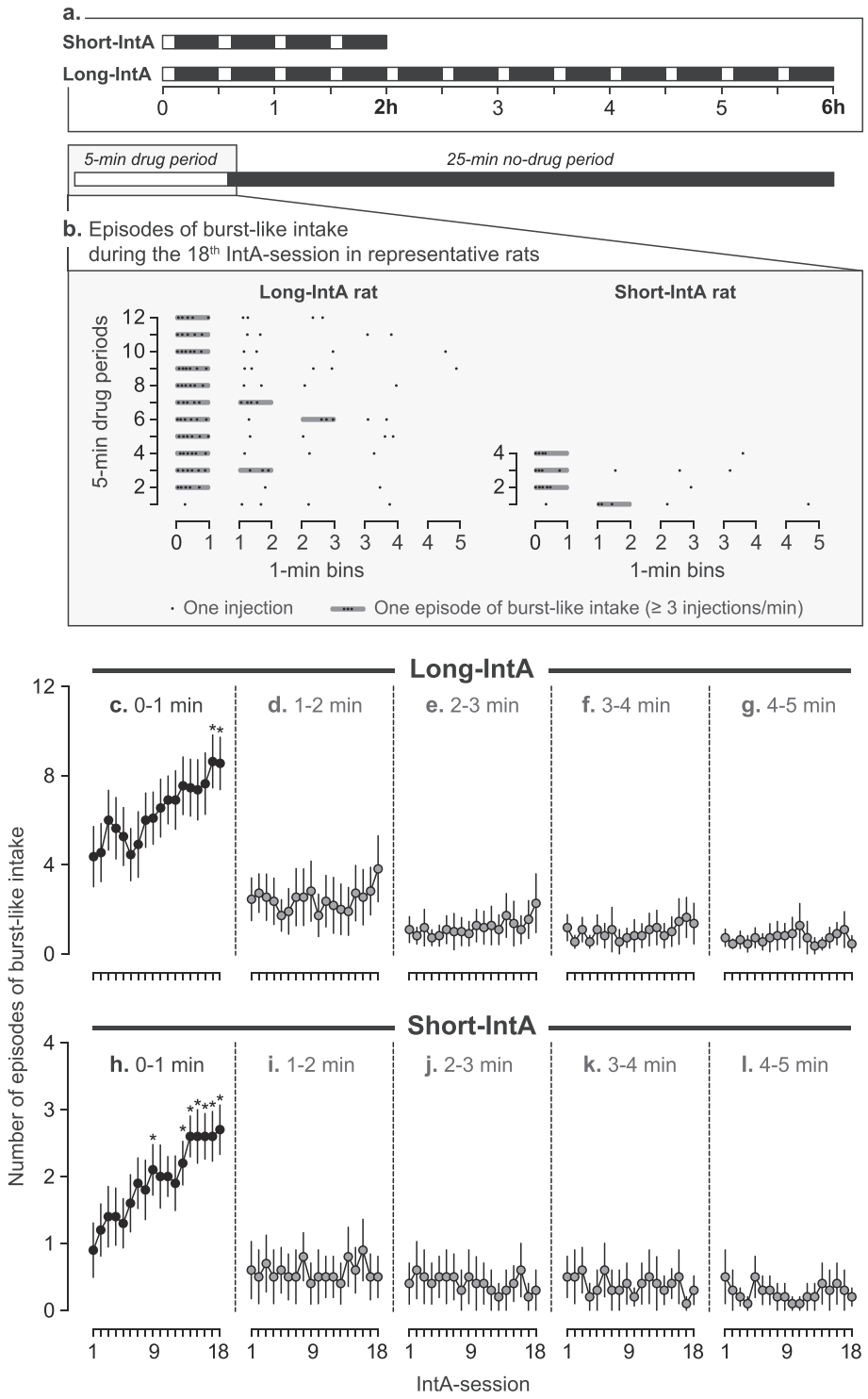

Figure 3 Rats self-administering cocaine during either extended (6 hours) or shorter (2 hours) IntA-sessions develop a burst-like pattern of cocaine use. (a) During each IntA-session, Long-IntA rats had twelve 5-minute drug periods, while Short-IntA rats had four 5-minute drug periods. Here, the 5-minute cocaine periods within each IntA-session were broken down into five 1-minute bins. (b) The pattern of cocaine intake during the 18th IntA-session is shown for a representative rat from each experimental group. Both rats took most of their injections in the first 1-minute bin of each 5-minute drug period. Both rats also showed episodes of burst-like intake (defined as taking ≥3 cocaine injections per 1-minute bin), in particular in the first minute of each 5-minute drug period. (c–g) and (h–l) show the number of episodes of burst-like intake during each minute of the 5-minute drug phases, across the 18 IntA-sessions, in Long-IntA and Short-IntA rats, respectively. *$P < 0.05$, versus first IntA-session. Data are mean ± SEM. n = 10–11/group

during extinction versus reinstatement (Group × Lever type × Session type, the latter two as within-subjects variables).

RESULTS

Extended intermittent-access sessions (6 hours) promote high and escalating levels of cocaine intake while shorter intermittent-access sessions (2 hours) produce low and stable levels of intake

Figure 2a shows average number of cocaine injections per IntA-session in rats given eighteen 2-hour (Short-IntA, S) or 6-hour (Long-IntA, L) self-administration sessions. Long-IntA rats self-administered more cocaine injections/session than Short-IntA rats (Main effect of Group, $F_{1,\ 19} = 6.84$, $P = 0.02$; Fig. 2a). The Long-IntA rats also escalated their cocaine intake between IntA-sessions (Main effect of IntA-session, $F_{17,\ 323} = 2.36$, $P = 0.002$; Group × IntA-session interaction effect, $F_{17,\ 323} = 1.65$, $P = 0.05$; Fig. 2a). From the 17th IntA-session on, Long-IntA rats self-administered more injections than on the first IntA-session (Bonfererroni's multiple comparisons test, all $P < 0.001$; Fig. 2a). In contrast, the Short-IntA rats maintained a stable level of drug intake between sessions (all $P > 0.05$; Fig. 2a). Figure 2b shows average number of injections/5-minute drug period. Both groups self-administered a similar average number of cocaine injections/5-minute drug period over the 18 self-administration sessions (Main effect of Group, $F_{1,\ 19} = 0.007$, $P = 0.93$; Fig. 2b). The number of these injections increased over IntA-sessions, and this escalation effect did not significantly vary as a function of group (Main effect of IntA-session, $F_{17,\ 323} = 2.43$, $P = 0.001$; Group × IntA-session interaction effect, $F_{17,\ 323} = 1.04$, $P = 0.41$; Fig. 2b). Thus, the escalation in overall cocaine intake observed specifically in the Long-IntA rats (Fig. 2a) is likely because, compared to Short-IntA rats, Long-IntA rats took slightly (but not significantly) more cocaine injections during each 5-minute drug phase of the latter IntA-sessions (average number of injections/5-minute drug phase in the 18th IntA-session ± SEM; Long-IntA rats, 9.2 ± 1.7; Short-IntA rats; 6.9 ± 1.2; Fig. 2b). Finally, Long-IntA rats took three times more cocaine than Short-IntA rats over the 18 IntA-sessions ($t_{19} = 2.62$, $P = 0.02$; Fig. 2c).

Rats self-administering cocaine during either extended (6 hours) or shorter (2 hours) intermittent-access sessions develop a burst-like pattern of cocaine use

Figure 3 shows episodes of burst-like cocaine intake in the two experimental groups. These episodes are defined as taking at least three injections per 1-minute bin, as adapted from (Belin *et al.* 2009; Allain *et al.* 2018). Here, the Long-IntA rats had twelve 5-minute cocaine periods/IntA-session and the Short-IntA rats had four 5-minute cocaine periods/IntA-session (Fig. 3a). As described previously in Long-IntA rats (Allain *et al.* 2018), both the Long-IntA and Short-IntA rats in the present study developed a burst-like pattern of drug use (Fig. 3b). To analyze episodes of burst-like intake, each 5-minute cocaine period was split into 1-minute bins. An episode of burst-like intake was counted when a rat took at least three cocaine injections within a 1-minute bin (see Fig. 3b for data from representative rats). Figures 3c–l show the number of episodes of burst-like intake during each minute of the 5-minute drug phases, across the 18 IntA-sessions. Figures 3c–g show these data in Long-IntA rats. Figures 3h–l show these data in Short-IntA rats. In both groups, episodes of burst-like intake occurred predominantly in the first 1-minute bin of each 5-minute cocaine period (Main effect of 1-minute bin, $F_{4,\ 40} = 25.98$; Figs. 3c–g; $F_{4,\ 36} = 26.44$; Figs. 3h–l; 1-minute bin × IntA-session interaction effect, $F_{68,\ 680} = 1.96$; Figs. 3c–g; $F_{68,\ 612} = 3.13$; Figs. 3h–l; all $P < 0.0001$). In both Long-IntA and Short-IntA rats, the number of episodes of burst-like intake in the first minute of each cocaine period also increased significantly between IntA-sessions ($F_{17,\ 170} = 3.05$; Fig. 3c; $F_{17,\ 153} = 4.57$; Fig. 3h; all $P \leq 0.0001$; no other comparisons were significant). In the Long-IntA rats, the number of episodes of burst-like intake on sessions 17–18 was greater than on the first IntA-session and, in the Short-IntA rats this effect was seen on sessions 9 and 13–18 (Bonferroni's multiple comparisons tests; all $P < 0.05$, Figs. 3c,h). In summary, both rats given Short-IntA and rats given Long-IntA sessions load up on cocaine at the start of each 5-minute cocaine period—self-administering cocaine at a rapid rate—and this burst-like effect sensitizes across IntA-sessions.

Rats self-administering cocaine during either extended (6 hours) or shorter (2 hours) intermittent-access sessions show a similar increase in drug-induced locomotion over sessions

Figures 4a,b show the pattern of cocaine intake (top two lines) and estimated brain cocaine concentrations for a representative rat from each group during the 1st and 18th IntA-sessions. In both rats, the IntA protocol produces a spiking pattern of estimated brain cocaine concentrations (Zimmer *et al.* 2011). Long-IntA rats self-administered more cocaine on the 18th session than on the 1st (Fig. 2a), and estimated brain cocaine concentrations also increased between the two sessions (Fig. 4a). However, Short-IntA rats took similar amounts of cocaine per session over time (Fig. 2a), thus estimated brain

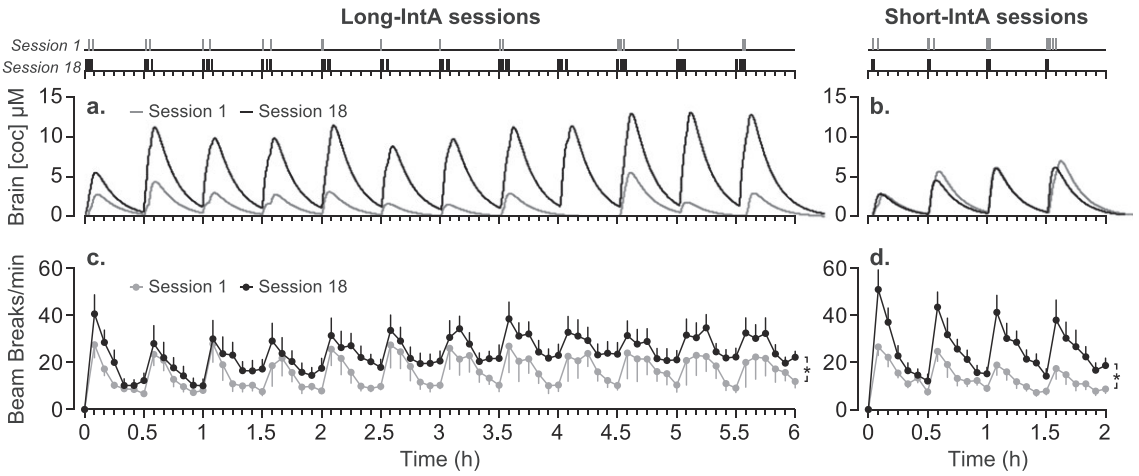

Figure 4 Rats self-administering cocaine during either extended (6 hours) or shorter (2 hours) IntA-sessions develop robust psychomotor sensitization. (a) and (b) show the pattern of cocaine intake and estimated brain cocaine concentrations as a function of time during the 1st (gray line) and 18th (black line) sessions, in a representative rat from each group. (c) and (d) show locomotor activity per min during the 1st (gray line) and 18th (black line) sessions, in Long-IntA and Short-IntA rats, respectively. *$P < 0.05$, versus first IntA-session. Data are mean ± SEM. n = 10–11/group

cocaine concentrations were similar between the 1st and the 18th sessions in these rats (Fig. 4b). Although brain cocaine concentrations increased over time in the Long-IntA rats but not in the Short-IntA rats, both groups showed greater cocaine-induced locomotor activity on the 18th session relative to the 1st (Main effect of Day, $F_{1,\ 10} = 5.74$, $P = 0.04$; Fig. 4c; $F_{1,\ 9} = 21.07$, $P = 0.001$; Fig. 4d). There were no group differences in cocaine-induced locomotor activity on the 1st IntA-session (Main effect of Group for the first 2 hours, $F_{1,\ 19} = 0.0008$, $P = 0.98$; gray lines; Figs. 4c,d) or on the 18th IntA-session (Main effect of Group for the first 2 hours, $F_{1,\ 19} = 1.65$, $P = 0.21$; black lines; Figs. 4c,d). Thus, the findings suggest that the two groups developed robust and similar levels of psychomotor sensitization to self-administered cocaine.

Rats that have self-administered cocaine during either extended (6 hours) or shorter (2 hours) intermittent-access sessions show similar levels of responding for cocaine under progressive ratio

While Long-IntA rats self-administered significantly more cocaine than Short-IntA rats during IntA-sessions, operant responding for cocaine under PR was similar in the two groups (Main effect of Group, $F_{1,\ 19} = 0.009$, $P = 0.92$; Fig. 5a). Both groups also lever-pressed more for higher doses of cocaine (Main effect of Dose, $F_{3,\ 57} = 23.64$, $P < 0.0001$; Dose × Group interaction effect, $F_{3,\ 57} = 1.92$, $P = 0.14$; Fig. 5a). Thus, rats that have previously taken cocaine during Short-IntA or Long-IntA sessions later show equivalent levels of incentive motivation for the drug.

Rats that have self-administered cocaine during either extended (6 hours) or shorter (2 hours) intermittent-access sessions show similar extinction of lever-pressing behavior

One month after PR testing, lever-pressing behavior was extinguished during a single 6-hour extinction session (Fig. 5b). During this session, all rats pressed more on the active than on the inactive lever (Main effect of Lever type, $F_{1,\ 19} = 12.98$, $P = 0.002$; Fig. 5b). Lever-pressing behavior also decreased over the extinction session (Main effect of Time, $F_{5,\ 95} = 15.13$, $P < 0.0001$; Fig. 5b), and there was a more pronounced decrease in pressing on the active versus the inactive lever (Lever type × Time interaction effect, $F_{5,\ 95} = 11.49$, $P < 0.0001$; Fig. 5b). There was no effect of group on lever-pressing behavior during the extinction session (Group × Lever type × Time interaction effect, $F_{5,\ 95} = 0.76$, $P = 0.58$; Fig. 5b).

Rats that have self-administered cocaine during either extended (6 hours) or shorter (2 hours) intermittent-access sessions show similar levels of cocaine-induced reinstatement

Immediately after the extinction session, rats were injected with 10 mg/kg cocaine i.p. and replaced in the operant test cages for a 2-hour reinstatement test. During this test, lever-pressing behavior was quantified but it produced no cocaine or cocaine cues. The i.p. cocaine injection reinstated lever-pressing behavior across the two groups (Main effect of Session type, $F_{1,\ 19} = 21.23$, $P < 0.0001$; Figs. 5c,d), and there were no group differences in this effect (Group × Session type interaction effect, $F_{1,\ 19} = 1.25$, $P = 0.28$;

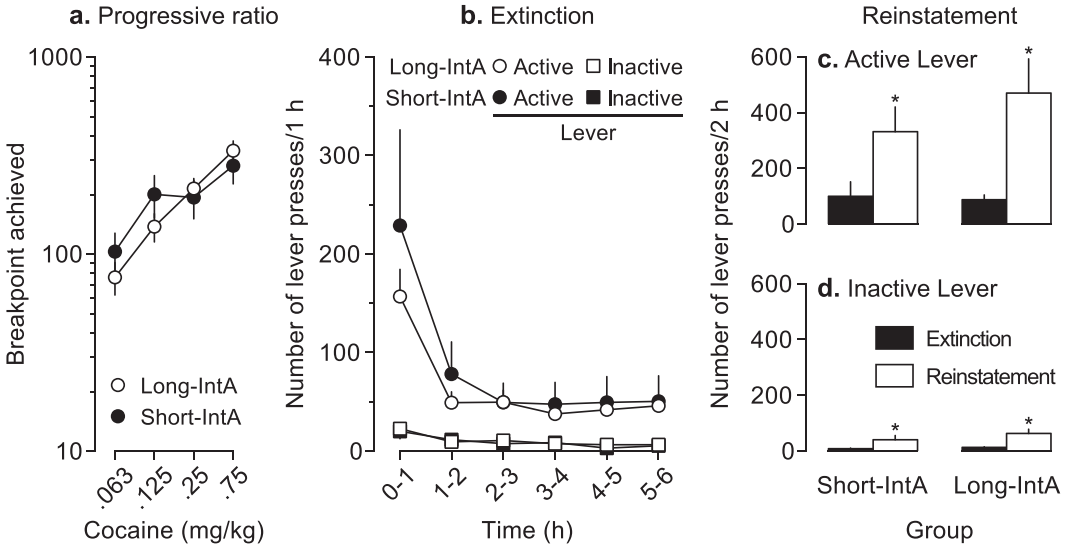

Figure 5 Rats with a history of self-administering cocaine during either extended (6 hours) or shorter (2 hours) IntA-sessions show (a) similar levels of incentive motivation for the drug and (b,c) similar levels of cocaine-primed reinstatement of extinguished drug-seeking behavior after 1 month of abstinence. In (a), Long-IntA and Short-IntA rats reached similar breakpoints for cocaine under a progressive ratio schedule of reinforcement. In (b), extinction of lever pressing behavior was similar in Long-IntA and Short-IntA rats. In (c), a priming injection of cocaine (10 mg/kg, i.p.) triggered similar levels of reinstatement of extinguished drug-seeking in the two groups. *$P < 0.05$, Main effect of Session type for each lever. Data are mean ± SEM. n = 10–11/group

Figs. 5c,d). During this reinstatement session, all rats pressed more on the levers than during the extinction session (Main effect of Session type, $F_{1,\ 19} = 19.05$, $P = 0.0003$, Fig. 5c; $F_{1,\ 19} = 16.91$, $P = 0.0006$, Fig. 5d), but both groups also pressed more on the active lever than on the inactive lever (2-way ANOVA on the reinstatement session only; Main effect of Lever type, $F_{1,\ 19} = 22.36$, $P = 0.0001$; Figs. 5c,d). There were no group differences in this effect (Group × Lever-type × Session type interaction effect, $F_{1,\ 19} = 0.99$, $P = 0.33$; Figs. 5c,d). In summary, rats that have previously taken cocaine during Short-IntA or Long-IntA sessions are equally susceptible to cocaine-induced reinstatement of drug-seeking behavior after 1 month of abstinence, at least at the cocaine dose tested (10 mg/kg, i.p.).

DISCUSSION

Here, we assessed the influence of IntA-session length on the development of addiction-like symptoms. We did this by allowing two groups of rats to self-administer cocaine daily during extended (6 hours) or shorter (2 hours) IntA-sessions (Zimmer *et al.* 2011; Zimmer *et al.* 2012). During IntA-sessions, cocaine was available for 5-minute periods interspersed with 25-minute no cocaine periods. This produces repeated spikes and troughs in estimated brain cocaine concentrations during each self-administration session [Figs. 4a,b; see also (Zimmer *et al.* 2011; Zimmer *et al.* 2012)]. Long-IntA rats had *twelve* 5-minute cocaine periods per session, and Short-IntA rats had *four* such cocaine periods per session. Only Long-IntA rats escalated their cocaine use, but both groups transitioned to an increasingly burst-like pattern of drug use, and both also developed robust psychomotor sensitization over time. After 18 IntA-sessions, the two groups showed similar incentive motivation to take cocaine (Fig. 5a). Finally, after 1 month of abstinence from cocaine, the two groups also showed equivalent levels of cocaine-primed reinstatement of extinguished drug-seeking (Fig. 5c).

Our findings have two major implications. First, continuously high and escalating levels of total cocaine intake are not necessary to evoke changes in behavior that are relevant to addiction [see also (Allain *et al.* 2018)]. Second, when cocaine is taken in an intermittent pattern, even short bouts of intake (2 hours/day) are sufficient to evoke an addiction-like phenotype. It must be considered that the present findings do not include a group that self-administered cocaine under continuous access conditions (i.e. a non-IntA comparison group). This being said, our results extend prior work showing that quite limited IntA experience is sufficient to see addiction-relevant plasticity. Calipari *et al.* (2015) showed that exposure to as little as three Long-IntA sessions with a 7-day abstinence period is enough to sensitize animals to both the incentive motivational effects of cocaine and the drug's effects at the dopamine transporter. It appears, therefore, that extended exposure to cocaine and/or escalation of drug use is not necessary to model in animals

the changes in brain and psychological function involved in addiction, at least when drug access is intermittent. This idea is also supported by our recent work, showing that high and escalating levels of cocaine intake are neither sufficient nor necessary to increase incentive motivation for the drug (Allain *et al.* 2018).

The present findings and others (Zimmer *et al.* 2012; Allain *et al.* 2018) bring to mind the contentious issue of whether most current drug policies might in some ways promote addiction, as they favor intermittent drug access. Clearly, unrestricted drug access would not be protective. Beyond the immediate consequences on health, extensive research has shown that animals given continuous access during long self-administration sessions do develop patterns of drug use that define addiction (Ahmed & Koob 1998, 1999; Paterson & Markou 2003; Mantsch *et al.* 2004). Instead, our findings provide new ways of thinking about the conditions that are sufficient and/or necessary for laboratory animals, and humans, to show addiction-like behaviors.

Prior work shows that imposing IntA to a reward can induce a 'frustration' effect in animals. This has most often been studied with food reward. With IntA to food reward, a 'frustration' effect can change the response to the reward itself, whereby animals will later binge on the reward after being exposed to cues that predict it [e.g. (Micioni Di Bonaventura *et al.* 2014)]. This 'frustration' can also lead to excessive consumption of other, more readily available rewards as a coping strategy [e.g. the schedule-induced polydipsia phenomenon (Falk, Neal, & Lau 1997)]. One explanation is that IntA to a reward produces a physiological/psychological state that results in exaggerated craving for that reward (Roper 1981). This could involve activation of physiological stress responses (Micioni Di Bonaventura *et al.* 2014). There is cross-sensitization between the effects of stress and the effects of drugs of abuse (Antelman *et al.* 1980; Herman, Stinus, & Le Moal 1984; Robinson, Angus, & Becker 1985). As such, stress-related mechanisms could be involved in the ability of intermittent cocaine access to both promote psychomotor sensitization [present findings and Allain *et al.* 2017] and evoke greater incentive motivation for the drug than continuous cocaine access (Zimmer *et al.* 2012; Allain *et al.* 2018).

Our findings suggest that the development of a burst-like pattern of cocaine use and the escalation of cocaine use over time are dissociable phenomena. The Short-IntA rats maintained stable levels of total cocaine intake between self-administration sessions, but just like Long-IntA rats, Short-IntA rats developed a burst-like pattern of cocaine use. Over time, rats in both groups began taking cocaine at a very rapid rate (≥3 injections/minute) each time they had access to the drug during IntA-sessions, and this effect sensitized across sessions (Fig. 3). That is, regardless of session length, the rats would load up on cocaine when brain levels were low (Allain *et al.* 2018). By imposing regular drug-free periods where brain cocaine levels drop, the IntA procedure might promote a recurring pattern of burst-like intake, where rats load up on cocaine each time it becomes available again during the session. Such episodes of high frequency cocaine intake are thought to contribute to the development of addiction (Belin *et al.* 2009; Martin-Garcia *et al.* 2014). Interestingly, the Short-IntA rats progressively developed a burst-like pattern of cocaine use, but they did not escalate their total cocaine intake over time (Fig. 2a). Thus, the development of burst-like use and escalation of cocaine intake over time might be symptoms of the transition to addiction that can emerge independently. In further support of this, IntA-rats can show recurring episodes of burst-like cocaine intake either with [(Allain *et al.* 2018) and Long-IntA rats here] or without escalating their total intake over time (Short-IntA rats here).

Both Long-IntA and Short-IntA rats developed robust psychomotor sensitization. Psychomotor sensitization is a long-lasting increase in drug-induced psychomotor activity in response to repeated drug exposure (Robinson & Berridge 1993). It is thought to reflect changes in the brain that also lead to sensitized drug wanting (Robinson & Berridge 1993; De Vries *et al.* 1998; Lorrain, Arnold, & Vezina 2000). However, in studies using continuous access to cocaine within each self-administration session, psychomotor sensitization and sensitization of drug wanting are often reported as being dissociable effects [(Ben-Shahar *et al.* 2004; Ben-Shahar *et al.* 2005; Ahmed & Cador 2006; Knackstedt & Kalivas 2007) but see (Ferrario *et al.* 2005)]. In contrast, IntA-rats develop robust psychomotor sensitization [present findings and (Allain *et al.* 2017)], and the degree of psychomotor sensitization predicts later incentive motivation for cocaine following IntA experience (Allain *et al.* 2017). In the present study, the Long-IntA rats escalated their cocaine intake over sessions. This could have contributed to the increase in drug-induced psychomotor activity over IntA-sessions. This being said, we do not believe that the increase in psychomotor activity over time is simply a consequence of increased drug use. The Short-IntA rats also showed robust psychomotor sensitization, even though they maintained stable total levels of cocaine intake between sessions. In addition, we have shown previously that Long-IntA rats prevented from escalating their intake still develop strong psychomotor sensitization (Allain *et al.* 2017).

CONCLUSION

In summary, rats given IntA to cocaine during either short or longer daily sessions develop an addiction

phenotype. This was indicated by the emergence of a burst-like pattern of cocaine use, the development of robust psychomotor sensitization, the willingness to self-administer cocaine in spite of increasing physical costs (as measured under PR), and significant cocaine-primed relapse during abstinence. Both Short-IntA and Long-IntA rats showed these behavioral effects, but only the Long-IntA rats had a history of escalated cocaine use. This suggests that taking high and escalating levels of cocaine is not necessary to change drug use over time, and that even short daily bouts of intermittent cocaine use are sufficient to evoke forms of neurobehavioral plasticity that are linked to the transition to addiction.

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Authors Contribution

F.A performed the experiments and analyzed the data. F.A and A.N.S designed the experiments and wrote the paper.

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