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ARTICLE

Amphetamine maintenance therapy during intermittent cocaine self-administration in rats attenuates psychomotor and dopamine sensitization and reduces addiction-like behavior

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D-amphetamine maintenance therapy shows promise as a treatment for people with cocaine addiction. Preclinical studies using Long Access (LgA) cocaine self-administration procedures suggest D-amphetamine may act by preventing tolerance to cocaine's effects at the dopamine transporter (DAT). However, Intermittent Access (IntA) cocaine self-administration better reflects human patterns of use, is especially effective in promoting addiction-relevant behaviors, and instead of tolerance, produces psychomotor, incentive, and neural sensitization. We asked, therefore, how D-amphetamine maintenance during IntA influences cocaine use and cocaine's potency at the DAT. Male rats self-administered cocaine intermittently (5 min ON, 25 min OFF x10; 5-h/session) for 14 sessions, with or without concomitant D-amphetamine maintenance therapy during these 14 sessions (5 mg/kg/day via s.c. osmotic minipump). We then assessed responding for cocaine under a progressive ratio schedule, responding under extinction and cocaine-primed reinstatement of drug seeking. We also assessed the ability of cocaine to inhibit dopamine uptake in the nucleus accumbens core using fast scan cyclic voltammetry ex vivo. IntA cocaine self-administration produced psychomotor (locomotor) sensitization, strong motivation to take and seek cocaine, and it increased cocaine's potency at the DAT. D-amphetamine co-administration suppressed the psychomotor sensitization produced by IntA cocaine experience. After cessation of D-amphetamine treatment, the motivation to take and seek cocaine was also reduced, and sensitization of cocaine's actions at the DAT was reversed. Thus, treatment with D-amphetamine might reduce cocaine use by preventing sensitization-related changes in cocaine potency at the DAT, consistent with an incentive-sensitization view of addiction.

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INTRODUCTION

Several pharmacological approaches are under study for treating cocaine addiction, but none is approved [1–3]. One promising strategy is to substitute cocaine with another dopaminergic agent [4, 5], such as D-amphetamine, but in a slower and longer-acting formulation. Low-dose D-amphetamine effectively decreases cocaine use in humans [6–10], nonhuman primates [10–15], and rats [16–19], with no or only transient effects on responding for food [11, 12, 14–16]. Although it is not clear how D-amphetamine produces these effects, it is not due to reduced brain cocaine concentrations, cross-tolerance or increases in cocaine's anxiogenic, or other toxic effects [12, 16]. Cocaine blocks dopamine uptake at the dopamine transporter (DAT) to enhance dopamine transmission and produce reward [20], and therefore, D-amphetamine might interfere with cocaine's actions at the DAT, thereby attenuating addiction-relevant effects [21–24].

Indeed, Siciliano et al. (2018) reported that, in rats, continuous, low-dose D-amphetamine (5 mg/kg/day for 14 d via s.c. mini-pump) reduces cocaine self-administration by preventing molecular changes that lead to a decrease (tolerance) in the ability of cocaine to inhibit dopamine uptake at the DAT in the nucleus accumbens core (NAcC) [25]. However, Long Access (LgA) cocaine self-administration procedures were used, which produce high and sustained brain cocaine concentrations [26–28]. In humans, cocaine use is typically more intermittent, which would produce peaks and troughs in brain cocaine concentrations [21, 29]. In rats, Intermittent Access (IntA) cocaine self-administration results in much less cumulative cocaine intake than LgA, but is more effective in producing addiction-like behaviors ([26–28, 30–35] Reviewed in [21, 22]). Of particular relevance here, LgA and IntA cocaine experience produce opposite effects on the dopamine system: tolerance versus sensitization, respectively, to cocaine-induced inhibition of dopamine uptake at the DAT and cocaine-induced dopamine overflow in the NAcC [35, 36]. Our objective here, therefore, was to assess the effects of D-amphetamine treatment during IntA cocaine self-administration on both addiction-relevant behaviors and cocaine's potency at the DAT. We assessed these effects after the cessation of D-amphetamine treatment/IntA cocaine experience because, (i) prior work in rats shows that D-amphetamine can suppress the motivation to take

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cocaine long after the cessation of treatment [16, 17, 25], potentially reducing the need for continuous D-amphetamine exposure for therapeutic efficacy, and (ii) this allowed for direct comparison with Siciliano et al. [25].

MATERIALS AND METHODS

See the Supplement for information on subjects, surgeries, drugs, and self-administration training. The animal care committee of the Université de Montréal approved all experimental procedures, and these complied with the guidelines of the Canadian Council on Animal Care.

Experiment 1: horizontal locomotor activity, cocaine-taking and -seeking

After intravenous catheter implantation and self-administration training [37], male Wistar rats (200–250 g; Charles River Laboratories, St Constant, QC) were assigned to a "COC + A" or "COC" group. A D-amphetamine-filled minipump (5 mg/kg/day; Sigma-Aldrich, Dorset, UK) was implanted subcutaneously in the COC + A rats ($n = 11$). COC rats received a saline-filled minipump ($n = 11$), or sham surgery ($n = 11$). All rats then self-administered cocaine (0.25 mg/kg/injection, delivered over 5 s; Medisca Pharmaceutique, St Laurent, QC) under a fixed ratio three schedule, for 14 IntA-sessions (Fig. 1a). Each 5-h session consisted of ten, 5-min cocaine-available periods separated by 25-min, no cocaine-available periods where levers were retracted [26]. We measured cocaine intake and locomotion during IntA-sessions.

The day after the last IntA-session, minipumps were removed. Sham rats in the COC group that did not have minipumps received a second sham surgery. This way, both experimental and control groups received the same number of surgeries. Two days later, we assessed responding for cocaine under a progressive ratio schedule of reinforcement (PR, Fig. 1a) [38].

After testing under PR, all COC + A and half of the COC rats were given 10, 2-h extinction sessions (1 session/day), and then five, 2-h pre-reinstatement sessions (see [39]) to further decrease the influence of cocaine cues on subsequent reinstatement testing [39–41]. During pre-reinstatement sessions, lever pressing no longer produced exteroceptive cocaine-associated cues.

After extinction and pre-reinstatement sessions (~3 weeks after discontinuation of D-amphetamine treatment), the rats were tested for cocaine-induced reinstatement of drug-seeking behavior (Fig. 1a). Sessions were similar to pre-reinstatement sessions, except that before each session, rats received cocaine i.p. (0, 7.5 and 15 mg/ml/kg, in ascending order, 1 dose/session, within-subjects). Cocaine seeking was measured as the number of presses on the previously cocaine-associated lever [42, 43]. Rats received an extinction session between reinstatement sessions.

We also compared the response to cocaine before and after D-amphetamine treatment, within-subjects. We used half of the COC rats for this (11/22 COC rats, consisting of 5 sham-operated rats and 6 rats previously with SAL-minipumps). PR scores were similar in the 11 COC rats given a 2nd round of D-amphetamine treatment and the 11 COC rats that completed extinction/reinstatement testing; Dose effect, $F_{2,40} = 36.23$, $p < 0.0001$; Group effect, $F_{1,20} = 0.001$, $p = 0.97$; Dose x Group effect, $F_{2,40} = 0.06$, $p = 0.94$; Fig. S1). After 14 IntA-sessions and PR testing, D-amphetamine-containing minipumps (5 mg/kg/day) were implanted in 11 COC rats, and these rats were given 14 additional IntA-sessions, now with concomitant D-amphetamine (Fig. 3a). The day after the last IntA-session, minipumps were removed. Two days later, the rats were tested under PR once again (Fig. 3a).

Experiment 2: cocaine's potency at the dopamine transporter

New rats self-administered cocaine or saline during 14 IntA-sessions. In the rats self-administering cocaine, one group received concomitant D-amphetamine treatment via minipump (5 mg/kg/day; $n = 7$), and a second group was sham-operated and remained D-amphetamine-naive ($n = 6$). Similarly, in the rats self-administering saline, one group received D-amphetamine ($n = 5$) and a second group remained D-amphetamine-naive ($n = 5$). This yielded four groups; "COC," "COC + A," "SAL," and "SAL + A" (Fig. 5a). The day after the last session, minipumps were removed (sham rats were sham operated). Five days later, brain sections were prepared for FSCV to measure cocaine-induced inhibition of dopamine uptake in the NAcC [44–46] (see Supplement). This approximates the 3–5-day delay between the last IntA-session and PR testing in Experiment 1.

Data analysis

Two-way repeated measures (RM) ANOVAs were used to determine effects on locomotion across IntA-sessions, and intake across PR sessions. Data on number of injections taken over IntA-sessions violated homoscedasticity and group differences on this measure were not analysed statistically. One-way RM ANOVAs were used to analyze escalation of intake over IntA-sessions in each group. To assess the relationship between cocaine intake during IntA-sessions versus during PR tests, we analysed goodness-of-fit (r^2) of the linear regression between the two variables. Three-way RM ANOVAs were used to analyse locomotion within IntA-sessions. Two-way RM ANOVA was used to determine effects on lever-presses during extinction, pre-reinstatement, and reinstatement sessions. When comparing responding before and with D-amphetamine in the same rats, one-way RM ANOVA was used to analyze cocaine intake and locomotion across IntA-sessions. Locomotion averaged over the 5-min cocaine periods was analyzed before and with D-amphetamine using paired *t*-tests (IntA-session 1 versus 14 and IntA-session 15 versus 28). Two-way RM ANOVA was used to compare responding under PR before and after D-amphetamine. Finally, two-way RM ANOVA was used to determine effects on dopamine overflow and apparent Km as a function of cocaine concentration. Significant interaction or main effects (P's < 0.05) were followed by Bonferroni's multiple comparisons' tests when appropriate.

RESULTS

Experiment 1a: effect of D-amphetamine maintenance on cocaine-induced horizontal locomotor hyperactivity, cocaine-taking, and -seeking

Intermittent access sessions. Sham rats and rats with a saline minipump did not differ on any of the behavioral measures, and therefore, were pooled to form one group ("COC"). There was greater variability in intake in the COC + A rats than in the COC rats (Levene's tests; $p < 0.05$), but on average the two groups self-administered a similar number of cocaine injections (see Fig. 1b). The greater variability in COC + A rats could result in part from the smaller number of rats in this group (11, versus 22 COC rats), and suppressed cocaine intake in some COC + A rats (see Fig. 2e–g). Still, on average, both groups escalated their intake over time (COC rats, $F_{13,273} = 5.06$, $p < 0.0001$, IntA-sessions 13 and 14 > IntA-sessions 2–7, all P's < 0.05; COC + A rats, $F_{13,130} = 2.15$, $p = 0.02$, IntA-session 13 >IntA-sessions 4–6, all P's < 0.05; Fig. 1b).

During the 1st IntA-session, locomotion was similar in COC and COC + A rats, but it increased over time only in COC rats, and by the 14th IntA-session, locomotion was greater in COC rats (Group × Session interaction effect, $F_{13,403} = 5.19$, $p < 0.0001$; Group effect, $F_{1,31} = 4.51$, $p = 0.04$; Bonferonni's tests, $p = 0.005$ at the 14th IntA-session; Fig. 1c). Increased locomotion over sessions in COC rats could involve increased cocaine intake over sessions. This is unlikely because neither the degree of escalation nor cumulative cocaine intake predicted this increase in locomotion ($r^2 = 0.12$ and $r^2 = 0.002$, respectively; All P's > 0.05; data not shown). We did not measure stereotypy and D-amphetamine might have decreased locomotion by promoting stereotypy [47]. However, continuous

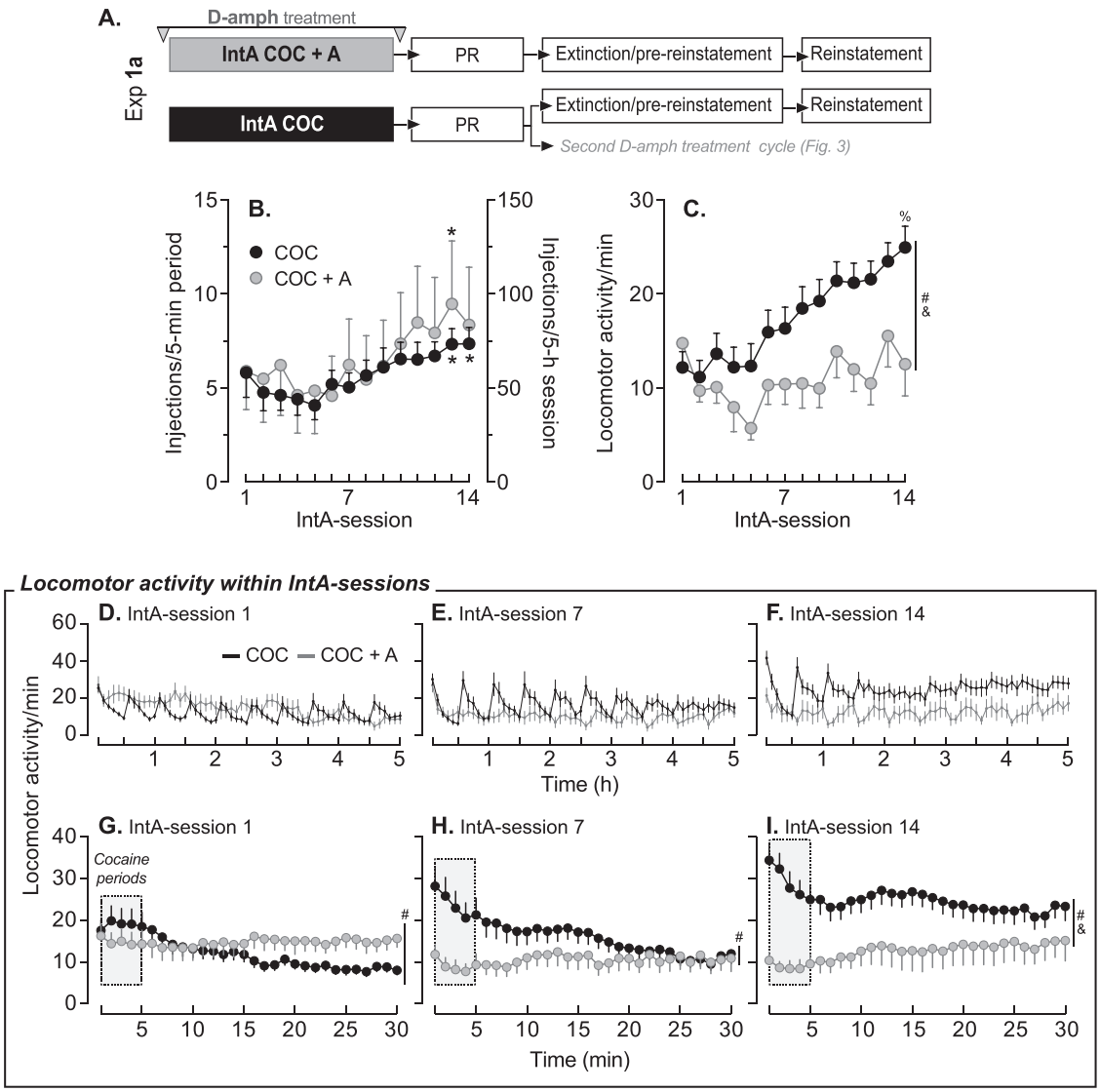

Fig. 1 D-amphetamine did not significantly influence cocaine intake, but it abolished both cocaine-induced psychomotor sensitization and spikes in locomotor activity during cocaine self-administration sessions. a In Experiment 1, rats self-administered cocaine under intermittent-access (IntA) conditions (COC). Some rats received D-amphetamine maintenance (COC + A; 5 mg/kg/day, via s.c. osmotic minipump) during intermittent cocaine self-administration. During D-amphetamine treatment/IntA cocaine self-administration, we assessed changes in cocaine intake and locomotor activity. After cessation of D-amphetamine treatment/IntA, we assessed responding for cocaine under a progressive ratio (PR) schedule of reinforcement, and cocaine-induced reinstatement of extinguished cocaine-seeking behavior. **b** The two groups took a similar number of cocaine injections, and both escalated their intake over time. **c** Horizontal locomotor activity/min increased over IntA-sessions only in COC rats, suggesting that only COC rats developed psychomotor sensitization to self-administered cocaine. **d–f** Intermittent cocaine intake produced spikes in locomotor activity during the 5-h IntA-sessions, and D-amphetamine suppressed this effect. **g–i** Locomotor activity/min averaged over the ten, 5-min cocaine (shaded in gray) and the ten, 25-min no cocaine periods of the 1st, 7th, and the 14th IntA-sessions. In COC rats only, locomotion increased over sessions, and was highest during the 5-min cocaine periods. Locomotion was also greater in COC versus COC + A rats. Data are mean ± SEM. $n = 22$ for the COC group, and $n = 11$ for the COC + A group. *P's < 0.05, versus IntA-sessions 2–7 in COC and versus IntA-sessions 4–6 in COC + A. $^{\%}p = 0.005$, COC versus COC + A. $^{\#}P$'s < 0.05, Group × Session or Group × Time interaction effect. $^{\&}P$'s < 0.05, main effect of Group. IntA, Intermittent Access. COC, Cocaine. A, D-amphetamine.

exposure produces significant tolerance to the stereotypy-inducing and other psychomotor activating effects of psychostimulant drugs [48–50]. Thus, we conclude that IntA cocaine self-administration produced psychomotor (locomotor) sensitization, as described previously (See also [27, 30, 37]), and D-amphetamine treatment prevented this sensitization.

D-amphetamine also produced qualitative changes in cocaine-induced locomotion. In COC rats, locomotion during IntA-sessions showed a spiking pattern, and D-amphetamine attenuated this (Fig. 1d–f). This is highlighted in Fig. 1g–i, where locomotion was averaged over the ten, 30-min cycles of cocaine-available (5 min; gray shading) and no cocaine-available (25 min) periods. In COC rats only, locomotion increased over sessions (Group x Session effect, $F_{2,62} = 13.96$, $p < 0.0001$; Main effect of Session in COC rats; IntA-session 7 > 1, $F_{1,21} = 10.91$, $p = 0.003$; IntA-session 14 > 1, $F_{1,21} = 63.72$, $p < 0.0001$; IntA-session 14 > 7, $F_{1,21} = 46.36$, $p < 0.0001$; Fig. 1g–i), and was highest during the 5-min cocaine periods (Group x Time effect, $F_{29,899} = 4.45$, $p < 0.0001$; Fig. 1g–i). Locomotion was also greater in COC versus COC + A rats, particularly from IntA-session 7 onwards (Group x Time interaction effect; $F_{29,899} = 4.92$, $p < 0.0001$, Fig. 1g; $F_{29,899} = 3.14$, $p < 0.0001$, Fig. 1h; $F_{29,899} = 1.54$, $p = 0.04$, Fig. 1i. Group effect; $F_{1,31} = 9.79$,

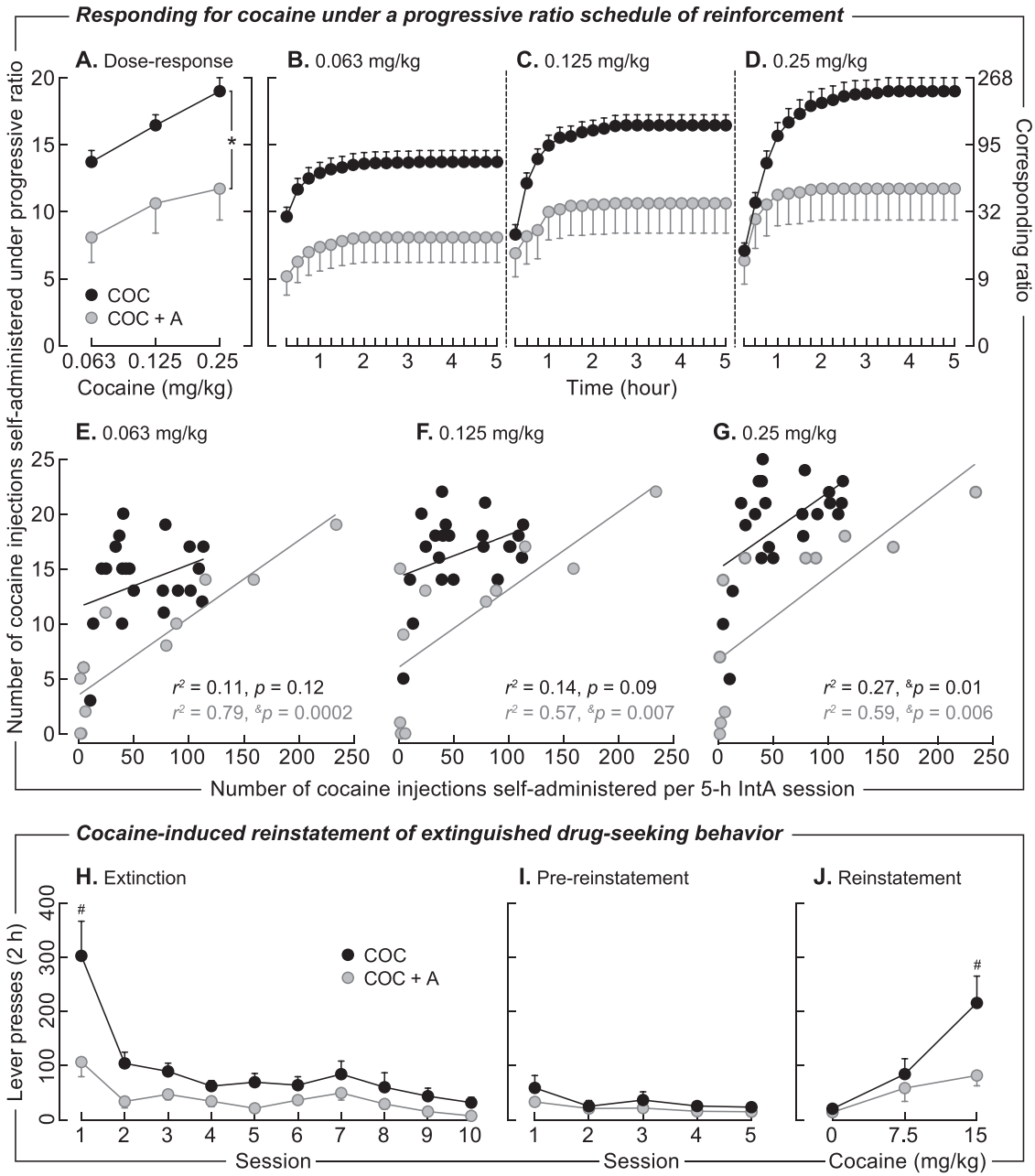

Fig. 2 D-amphetamine maintenance during intermittent cocaine self-administration decreased subsequent cocaine-taking and -seeking. **a** During progressive ratio tests, responding for cocaine was dose-dependent, and COC + A rats responded less for cocaine compared with COC rats. **b–d** Cumulative number of self-administered cocaine injections (left *y*-axis) and corresponding ratio (right *y*-axis) during each 5-h progressive ratio test, as a function of cocaine dose. Correlations between the average number of cocaine injections taken per 5-h IntA-session and number of cocaine injections earned during progressive ratio tests at 0.063 mg/kg/infusion (**e**), 0.125 mg/kg/infusion (**f**) and 0.25 mg/kg/infusion (**g**) cocaine. Cocaine intake during IntA-sessions predicted responding for all doses of cocaine tested under a progressive ratio schedule in COC + A rats, but not in COC rats. **h** Under extinction conditions, COC + A rats showed less cocaine-seeking behavior than COC rats, and both groups extinguished responding over time. **i** During pre-reinstatement sessions (additional extinction sessions, but where cocaine cues were no longer presented) both groups decreased their active-lever pressing over sessions with no group differences. **j** COC + A rats were less vulnerable to cocaine-induced reinstatement of extinguished cocaine-seeking than COC rats were, in particular after a 15 mg/kg cocaine prime. $^{*}p < 0.01$, main effect of Group. $^{\&}p \leq 0.01$, non-zero slope. $^{\#}p < 0.01$, versus COC + A. Data are mean ± SEM. (**a–g**) $n = 22$ for the COC group, and $n = 11$ for the COC + A group. (**h–j**) *n*'s = 10–11/group. IntA, Intermittent Access. COC, Cocaine. A, D-amphetamine.

$p = 0.004$; Fig. 1i; No other comparisons were significant). Thus, D-amphetamine prevented psychomotor sensitization to cocaine and changed the temporal kinetics of cocaine-induced locomotion.

Responding under a PR schedule. D-amphetamine treatment was discontinued after the 14th IntA-session. Two days later, rats received PR tests. Both groups responded more for higher cocaine doses (Dose effect, $F_{2,62} = 34.11$, $p < 0.0001$; Group × Dose effect, $F_{2,62} = 1.37$, $p = 0.26$; Fig. 2a), and COC rats earned more cocaine than COC + A rats (Group effect, $F_{1,31} = 11.24$, $p = 0.002$; Fig. 2a). Figure 2b–d further illustrates this, showing cumulative responding for cocaine during PR sessions. COC rats also persisted in responding for cocaine for longer than COC + A rats (Group effect

on session duration, $F_{1,31} = 8.51$, $p = 0.007$; data not shown). Thus, under PR, COC rats persevered in working for cocaine as cost in effort increased, more so than COC + A rats did. This suggests that prior D-amphetamine maintenance reduced subsequent incentive motivation for cocaine.

In COC rats, the amount of prior cocaine intake during IntA-sessions did not predict subsequent responding for cocaine under a PR schedule at 0.063 and 0.125 mg/kg/infusion cocaine ($r^2 = 0.11$ and 0.14, respectively, P's > 0.05; Fig. 2e, f; see also [27, 28, 51]), but at the highest cocaine dose tested during PR (0.25 mg/kg/infusion) less cocaine intake during previous IntA-sessions predicted less responding on the PR schedule ($r^2 = 0.27$, $p = 0.01$; Fig. 2g). This extends the idea that cocaine consumption and appetitive responding for cocaine can be dissociable [26, 28, 51–53]. However, in COC + A rats, less cocaine intake during IntA-sessions did predict lower responding during PR tests, at all cocaine doses tested (All r^2 coefficients ≥ 0.57; All P's < 0.01; Fig. 2e–g). The degree of escalation during IntA (difference between the number of cocaine injections taken on the 13th versus 4th IntA-session) did not predict the number of injections taken under PR for COC rats (All r^2 coefficients ~ 0; All P's > 0.05; Fig. S2) but it did so in COC + A rats (All r^2 coefficients ≥ 0.39; All P's < 0.05; Fig. S2). Thus, while D-amphetamine did not reduce average cocaine intake during IntA-sessions (Fig. 1b), the amount of cocaine taken and the degree of escalation of cocaine intake while D-amphetamine was onboard predicted later responding for cocaine under a PR schedule.

Extinction. Both groups decreased responding over the extinction sessions (Session effect, $F_{9,171} = 15.37$, $p < 0.0001$; Fig. 2h), but COC rats responded more than COC + A rats did (Session × Group effect, $F_{9,171} = 3.87$, $p = 0.0002$; Group effect, $F_{1,19} = 10.41$, $p = 0.004$; Fig. 2h), especially on the 1st session (Bonferroni's tests, 1st session; $p < 0.0001$. All other P's > 0.05; Fig. 2h). This suggests that COC rats attributed more incentive value to cocaine, to the cocaine-paired cues, or both. After extinction sessions, rats received 5 'pre-reinstatement' sessions, where active-lever presses no longer produced cocaine cues. Both groups decreased their active-lever pressing even more over these sessions (Session effect, $F_{4,76} = 5.77$, $p = 0.0004$; Fig. 2i), and there were no group differences (Group effect, $F_{1,19} = 0.98$, $p = 0.33$; Group × Session effect, $F_{4,76} = 0.83$, $p = 0.51$; Fig. 2i).

Cocaine-induced reinstatement. Priming injections of cocaine dose-dependently increased active-lever presses in both groups (Dose effect, $F_{2,38} = 19.26$, $p < 0.0001$; Fig. 2j), but to a greater extent in COC than in COC + A rats (Group × Cocaine dose effect, $F_{2,38} = 5.24$, $p = 0.01$; Fig. 2j), particularly after a 15 mg/kg cocaine prime (Bonferroni's tests, $p = 0.003$; all other P's > 0.05; Fig. 2j). Thus, D-amphetamine treatment during IntA cocaine self-administration decreased later vulnerability to cocaine-induced reinstatement of extinguished drug-seeking behavior, long after the cessation of treatment.

Experiment 1b: D-amphetamine maintenance after a history of intermittent cocaine intake

After 14 IntA-sessions (Fig. 1) and testing under a PR schedule (Fig. 2a–d), 11 COC rats received 14 additional IntA-sessions (sessions 15–28), now with concomitant D-amphetamine (Fig. 3a). D-amphetamine was then discontinued and responding for cocaine under a PR schedule was assessed again. Rats escalated their intake over the 28 IntA-sessions ($F_{27,270} = 3.47$, $p < 0.0001$; Bonferroni's test, IntA-session 1 versus 28, $p = 0.02$; Fig. 3b). Cocaine-induced locomotion increased over the first 14 IntA-sessions (i.e., without D-amphetamine; Bonferroni's test; IntA-session 1 versus 14, $p < 0.0001$; Fig. 3c), but there was no further increment in locomotion over IntA-sessions 15–28, when rats now received D-amphetamine ($p = 0.66$; Fig. 3c). Figure 3d, e further highlight this, showing that when locomotion is averaged over the 5-min cocaine periods, locomotion increases between IntA-session 1 and 14 ($t_{10} = 3.92$, $p = 0.003$; Fig. 3d), and decreases between IntA-session 15 and 28 ($t_{10} = 2.37$, $p = 0.04$; Fig. 3e). Before D-amphetamine treatment, locomotion also followed a spiking pattern during IntA-sessions (Fig. 3f–h). D-amphetamine attenuated this (Fig. 3i–k). Thus, D-amphetamine attenuated further psychomotor sensitization to cocaine, even when given after prior IntA cocaine self-administration experience in the absence of amphetamine.

Responding under a PR schedule. Before and after D-amphetamine treatment, rats responded more for higher cocaine doses during PR tests (Dose effect, $F_{2,20} = 79.03$, $p < 0.0001$; Fig. 4a). Rats earned fewer cocaine injections after D-amphetamine treatment than before (Treatment effect, $F_{1,10} = 16.51$, $p = 0.002$; Dose × Treatment effect, $F_{2,20} = 0.42$, $p = 0.66$; Fig. 4a, see Fig. 4b–d for individual values). After D-amphetamine treatment, cumulative cocaine intake during PR sessions was also decreased (Fig. 4e–g). Thus, although rats escalated their cocaine intake even further under D-amphetamine (IntA-sessions 15–28, see Fig. 3b), and they more than doubled their average cumulative cocaine exposure with these additional sessions (average cumulative cocaine intake; IntA-sessions 1–14; 183 mg/kg ± 41 SEM; IntA-sessions 1–28; 463 mg/kg ± 89 SEM), they responded less for cocaine under a PR schedule after D-amphetamine treatment. This suggests that while D-amphetamine might not prevent escalation of cocaine intake, it decreases the amount of physical effort rats are willing to expend to obtain cocaine. This is unlikely due to repeated testing, because with repeated testing, responding for cocaine under a PR schedule remains stable or even increases [54] (see also [32]).

Experiment 2: D-amphetamine maintenance effects on cocaine's potency at the dopamine transporter

COC and COC + A rats took similar amounts of cocaine, and D-amphetamine treatment increased saline self-administration (Fig. S3; unpaired t tests, COC versus COC + A, $t_{11} = 0.55$, $p = 0.59$; SAL versus SAL + A, $t_8 = 2.77$, $p = 0.02$). As in Experiment 1, locomotion followed a spiking pattern during cocaine self-administration sessions, increased across sessions (Fig. 5b, black curves), and D-amphetamine blunted both effects (Fig. 5b, gray curves). In rats self-administering saline (Fig. 5c), D-amphetamine initially increased locomotion, but the SAL and SAL + A groups had similar locomotor counts by the last (14th) session.

Five days after cessation of IntA cocaine self-administration/D-amphetamine treatment, cocaine-induced inhibition of DA uptake in the NAcC was measured using ex vivo FSCV. Figure 5d shows representative FSCV traces following bath-applied cocaine (see Fig. S4 for all traces at baseline and after bath-application of 0.3 µM cocaine). Increasing cocaine concentrations enhanced stimulated dopamine overflow, and this did not differ between groups (Percent of 0.3 µM cocaine, Concentration effect, $F_{4,72} = 36.97$, $p < 0.0001$; Group effect, $F_{3,18} = 0.26$, $p = 0.85$; Fig. 5e; Percent of SAL, Concentration effect, $F_{4,44} = 1.25$, $p = 0.3$; Group effect, $F_{1,11} = 0.23$, $p = 0.64$; Fig. 5f). As reported previously [35], 30 µM cocaine did not significantly alter stimulated dopamine overflow, in any group. Increasing cocaine concentrations also augmented dopamine uptake inhibition (Concentration effect, $F_{4,72} = 126.7$, $p < 0.0001$; Fig. 5g), and the magnitude of this effect varied as a function of group (Group × Concentration effect, $F_{12,72} = 2.65$, $p = 0.005$; Fig. 5g; $F_{4,44} = 3.56$, $p = 0.01$; Fig. 5h). At 30 µM cocaine, dopamine uptake inhibition was greatest in COC rats (Bonferroni's tests; all P's < 0.05; Fig. 5g, h; See Fig. S5 for representative traces and pseudo-color plots). Thus, IntA experience increased cocaine's potency at the DAT, as reported previously [31, 35]. Importantly, D-amphetamine prevented this neurochemical sensitization. In cocaine-naive, SAL rats, D-amphetamine did not change cocaine-induced dopamine uptake inhibition (D-amphetamine effect, $F_{1,7} = 0.01$, $p = 0.1$;

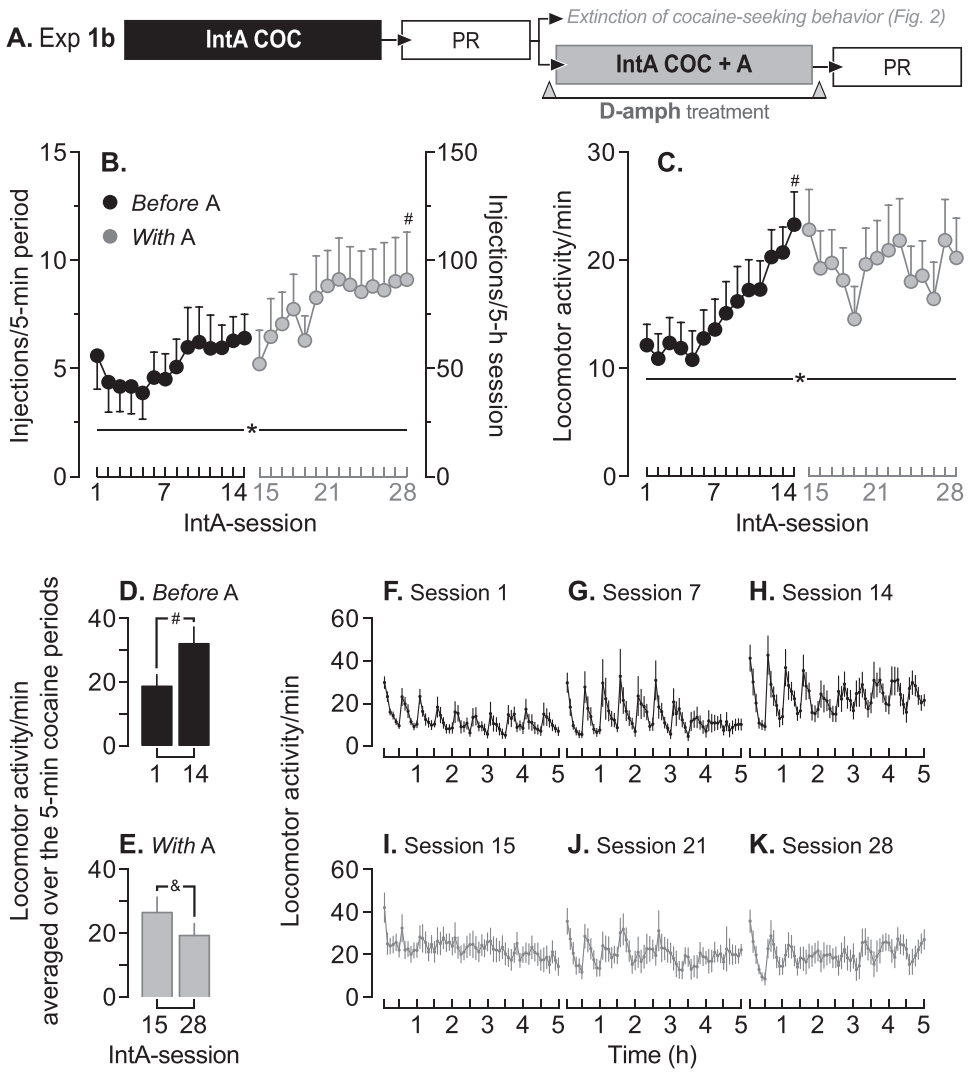

Fig. 3 D-amphetamine maintenance does not prevent the escalation of cocaine intake in already cocaine-experienced rats. a In Experiment 1, a subset of COC rats was used to compare responding for cocaine under a PR schedule before and after D-amphetamine treatment, in a within-subjects design. Thus, after 14 initial IntA-sessions without D-amphetamine (IntA-sessions 1–14) and PR tests, these rats were given 14 more IntA-sessions now with concomitant D-amphetamine treatment (IntA-sessions 15–28). After the 28th IntA-session, D-amphetamine treatment was ceased, and rats received a second set of PR tests. **b** Before and with D-amphetamine treatment, rats escalated their cocaine intake over IntA-sessions. **c** Locomotor activity increased over intermittent cocaine self-administration sessions, and it stabilized with D-amphetamine (IntA-session 15 versus 28). Locomotor activity/min averaged over the 5-min cocaine periods (**d**) increased from the 1st to the 14th IntA-session (before D-amphetamine) and (**e**) decreased from the 15th to the 28th IntA-session, when rats now received concomitant D-amphetamine treatment. **f**–**h** Locomotor activity during intermittent cocaine self-administration sessions followed a spiking pattern before D-amphetamine, and (**i**–**k**) D-amphetamine attenuated this spiking effect. Data are mean ± SEM. $n = 11$. *P's < 0.0001, main effect of Session. #P's < 0.05, versus IntA-session 1. &$p < 0.05$, versus IntA-session 15. IntA, Intermittent Access. A, D-amphetamine.

D-amphetamine × Concentration effect, $F_{4,28} = 0.45$, $p = 0.77$; Fig. 5g; See also [25, 47]). Thus, D-amphetamine normalized cocaine potency at the DAT by reversing cocaine-induced neurochemical sensitization, without changing cocaine's neurochemical effects in control rats.

DISCUSSION

Rats self-administered cocaine on an IntA schedule, with or without D-amphetamine maintenance treatment, and we assessed the development of psychomotor sensitization and addiction-like behaviors. These behaviors included high motivation for cocaine, as measured by responding for the drug under a PR schedule [38], resistance to extinction, and drug-induced reinstatement of extinguished drug-seeking behavior. Finally, the ability of cocaine to inhibit DA uptake in the NAcC was assessed ex vivo. We report three main findings. First, IntA cocaine self-administration induced psychomotor (locomotor) sensitization, and this was attenuated by D-amphetamine treatment, despite no effect on average cocaine consumption. Second, in both previously cocaine-naive and cocaine-experienced rats, D-amphetamine treatment decreased responding for cocaine both under a PR schedule of reinforcement and during extinction sessions, and reduced cocaine-primed reinstatement of extinguished drug-seeking behavior, relative to rats that received cocaine alone. Third, IntA experience enhanced cocaine's potency at the DAT, and D-amphetamine prevented this effect. Thus, D-amphetamine treatment during IntA cocaine self-administration may reduce subsequent motivation for cocaine by interacting with the DAT to prevent sensitization-related changes in cocaine potency and dopamine-mediated signaling.

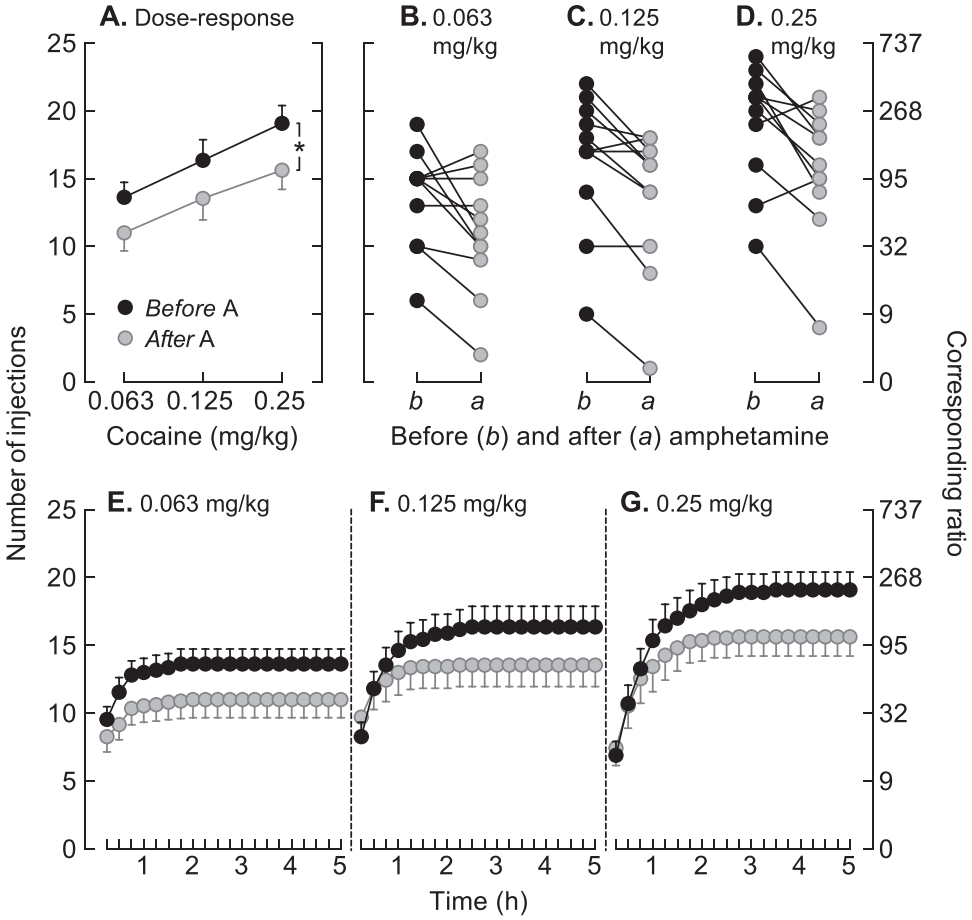

Fig. 4 In cocaine-experienced rats, D-amphetamine maintenance during intermittent cocaine self-administration decreases later responding for cocaine under a progressive ratio schedule of reinforcement. a Rats earned less cocaine injections after versus before D-amphetamine treatment. **b**–**d** Responding for cocaine under progressive ratio in individual rats before (b) and after (a) D-amphetamine treatment. **e**–**g** Cumulative number of self-administered cocaine injections (left *y*-axis) and corresponding ratio (right *y*-axis) during each 5-h progressive ratio test, as a function of cocaine dose. Data are mean ± SEM. $n = 11$. *$p = 0.002$, main effect of Treatment. A, D-amphetamine.

Long access versus intermittent access

D-amphetamine maintenance therapy can decrease cocaine use in people with addiction [6–9], and so the neurobiological and psychological basis of this effect is of interest. In an earlier study addressing this question Siciliano et al. (2018) trained rats to self-administer cocaine for 6 h/day (LgA) [25]. Many studies show that LgA is especially effective in producing addiction-like behaviors, relative to rats self-administering 1–2 h/day (Short Access, ShA). These behaviors include escalation of cocaine intake, high motivation for the drug, a high propensity to reinstate extinguished cocaine-seeking behavior and continued drug-seeking in the face of an adverse consequence [26, 55–58]. It is also reported that LgA experience produces tolerance in the ability of cocaine to inhibit the DAT [35, 59, 60]. Importantly, Siciliano et al. (2018) found that D-amphetamine treatment not only prevented and reversed the escalation of cocaine intake and the increase in motivation for cocaine otherwise produced by LgA experience, but also prevented the tolerance to cocaine's effect on the DAT produced by LgA [25]. They proposed, therefore, that D-amphetamine attenuated addiction-like behavior because it reversed the tolerance produced by extended cocaine use [25]. This is consistent with the view that addiction results from tolerance-related adaptations in the DA system, whereby drug-seeking and drug-taking behavior is motivated primarily to overcome this "DA deficiency" and associated anhedonia [61, 62].

However, recent studies using IntA self-administration procedures have begun to paint a very different picture of how cocaine use changes brain and behavior to promote addiction [26–28, 30–35, 63] (Reviewed in [21, 22]). During LgA, brain cocaine concentrations are maintained at a steady high level during each self-administration session, and the resultant large amount of cocaine consumption was thought to be necessary for the development of addiction-like behavior [55, 64, 65]. It turns out this is not the case. Zimmer et al. (2011) initially developed the IntA cocaine-self-administration procedure [66], which results in an intermittent, "spiking" pattern in brain cocaine concentrations, because it was thought to better model patterns of cocaine use in humans [29, 67]. Despite much less total cocaine consumption than LgA (comparable to ShA conditions) IntA experience not only also produces escalation of cocaine intake, but is either more effective, or at least as effective, as LgA in producing the addiction-like behaviors described above [26–28, 30, 32–34, 36] (Reviewed in [21, 22]). Furthermore, rather than producing tolerance, IntA experience enhances (sensitizes) cocaine's potency in inhibiting DA uptake ex vivo [31, 35], and cocaine-induced DA overflow in vivo [36]. This is consistent with the behavioral and neurobiological effects of experimenter-administered cocaine when given continuously versus intermittently [68, 69].

Given the dramatic differences in the effects of LgA versus IntA on the DAT, it was important to determine the effects of D-amphetamine treatment on addiction-like behavior and DAT function in rats with IntA cocaine experience. D-amphetamine treatment attenuated addiction-like behavior produced by intermittent cocaine intake, as indicated by reductions in motivation

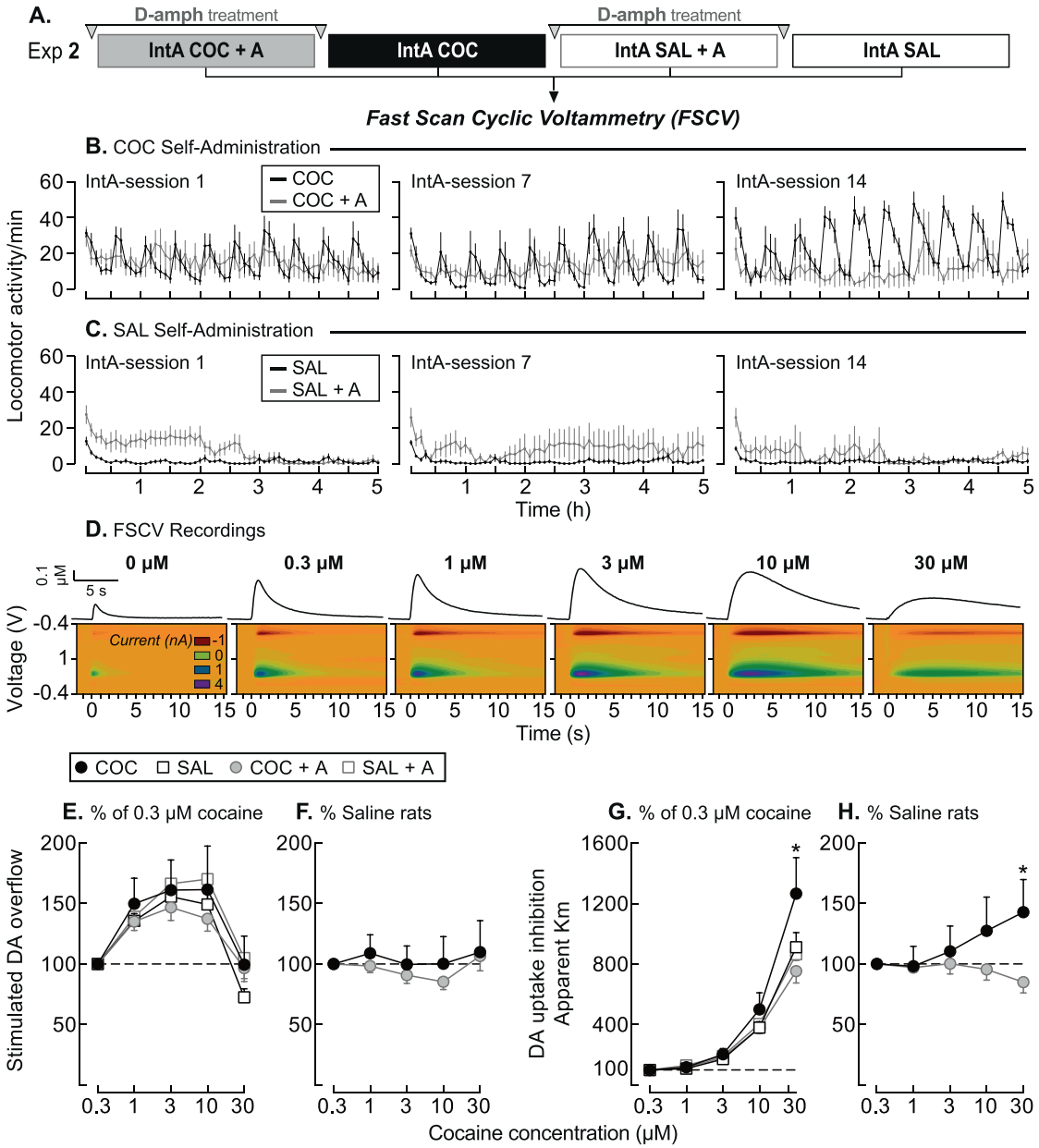

Fig. 5 D-amphetamine maintenance during intermittent cocaine intake prevents sensitization to cocaine's effects at the dopamine transporter. a In Experiment 2, rats self-administered cocaine (COC) or saline (SAL), with or without concomitant D-amphetamine treatment (COC + A and SAL + A). After cessation of D-amphetamine treatment/IntA experience, we used fast-scan cyclic voltammetry (FSCV) to assess cocaine-induced dopamine reuptake inhibition at the dopamine transporter in the nucleus accumbens core. **b** As in Experiment 1, intermittent cocaine intake produced spikes in locomotor activity, and spike amplitude increased with more extensive cocaine-taking experience. D-amphetamine attenuated both the locomotion spikes and the increase in spike amplitude over time. **c** D-amphetamine initially increased locomotion in rats self-administering saline, but this effect abated by the last (14th) session. **d** Representative traces from a SAL rat showing relative extracellular dopamine levels over time evoked by bath-application of different cocaine concentrations in the nucleus accumbens core. Fast-scan cyclic voltammetry heat maps from the same SAL rat illustrate the current (color in the *z*-axis) as a function of the potential applied to the electrode (*y*-axis) and time (*x*-axis). Pseudo-color plots were magnified to highlight dopamine spikes. The position of oxidative and reduction currents in the pseudo-color plots identify the oxidized substance as dopamine. **e**, **f** Electrically-evoked dopamine overflow (analysed as dopamine peak height) increased as a function of cocaine concentration, with no group differences. **g**, **h** % of apparent Km as a function of cocaine dose preferentially increased in COC rats and this increase is prevented in COC + A rats. The dose of 30 µM cocaine more effectively inhibited dopamine uptake in COC rats than in the other groups. In rats previously naive to cocaine (SAL), D-amphetamine did not change cocaine-induced dopamine uptake inhibition. Because in **e** and **g**, SAL and SAL + A rats were similar in all measures, they were pooled together in **f** and **h**. Data are mean ± SEM. $n = 4$–7/group. *$p < 0.05$, COC versus all other groups. IntA intermittent access, DA dopamine, COC cocaine, SAL Saline. A, D-amphetamine.

for cocaine, responding during extinction and in the magnitude of cocaine-induced reinstatement of cocaine seeking. D-amphetamine was efficacious both in previously cocaine-naive rats and in cocaine-experienced rats, suggesting that D-amphetamine can suppress both the development and the expression of addiction-like behavior. In the present experiments motivation to take and seek cocaine was assessed after D-amphetamine treatment cessation, but effects might be different while D-amphetamine is onboard. In

clinical trials, the effects of D-amphetamine maintenance on cocaine use are generally assessed while D-amphetamine is onboard (e.g., [9, 10]), and it is not known how long effects might persist after treatment cessation. Studies in rats suggest that the ability of D-amphetamine maintenance to reduce cocaine versus food choice might dissipate 1 week after treatment cessation [18]. However, consistent with our findings, D-amphetamine treatment in rats can suppress the motivation to take cocaine both during treatment and for up to 2 weeks post-treatment ([16, 17]; See also [25]). These findings suggest potentially persistent effectiveness, even without daily maintenance, and this issue should be examined in the clinic.

We found that D-amphetamine did not change the effects of a broad range of cocaine concentrations (1–30 µM) on electrically-evoked dopamine overflow or on cocaine's potency at the DAT in cocaine-naive rats (See also [25, 47]), but D-amphetamine prevented the sensitization of cocaine's action at the DAT produced by IntA. Our results concord with the observation that following cocaine self-administration experience, "…*alterations in cocaine potency and rescue by D-amphetamine treatment occur independently from canonical DAT function*…" (p. 489; [25]). Although speculative, one possibility is that D-amphetamine promotes post-translational modifications to the DAT, including changes in DAT phosphorylation [70]. We hypothesize, therefore, that D-amphetamine reduced the high motivation for cocaine produced by IntA experience by preventing *sensitization* to cocaine's effects at the DAT. This is consistent with an incentive-sensitization view of addiction [71].

How can both an increase and decrease in DAT function attenuate addiction-like behavior?

D-amphetamine treatment reduces the development of addiction-like behavior produced by both LgA [25] and IntA cocaine self-administration experience (present findings), but produces apparently opposite effects on DAT function under these two conditions. One possibility is that at least some of the addiction-like behaviors produced by LgA versus IntA experience are due to drug-induced changes in different neuropsychological processes [33]. For example, the escalation of intake and the high level of effort expended to obtain cocaine could be due to tolerance to cocaine's desired effects in the case of LgA, but to sensitization of drug "wanting" in the case of IntA [22, 33, 36]. Two lines of evidence support this. First, IntA produces sensitization to the locomotor activating, incentive motivational and dopamine-increasing effects of cocaine [26–28, 30, 31, 33, 35–37, 51, 63], whereas LgA can *decrease* the locomotor activating effects of cocaine and dopamine function, at least soon after the discontinuation of LgA [35, 59, 60, 72]. Note that psychomotor sensitization may be expressed after a long period of withdrawal from LgA [57, 63]. Second, D-amphetamine treatment reduced the escalation of cocaine intake under LgA conditions [25], but not during IntA (present study), further suggesting that different processes are involved.

D-amphetamine reduced incentive motivation for cocaine, but on average it did not affect the escalation of cocaine intake during IntA-sessions. This was surprising given that D-amphetamine suppressed the development of psychomotor sensitization across IntA-sessions. This could indicate that escalation of cocaine intake during IntA experience does not necessarily reflect sensitization of an appetitive process, and that cocaine consumption and appetitive responding for the drug are dissociable [26, 28, 51–53]. Indeed, we found that cocaine intake during IntA-sessions did not predict responding for the drug under a PR schedule (see also [28, 30, 37]). Alternatively, perhaps D-amphetamine's therapeutic effects are most robust after the cessation of treatment, because rats can take enough cocaine during IntA-sessions to overwhelm any effect of ongoing D-amphetamine treatment on the DAT. The implication is that D-amphetamine maintenance reduces incentive motivation for cocaine by inoculating against cocaine-induced, sensitization-related plasticity at the DAT. Future work should also examine behavioral and neurochemical outcomes at different timepoints after D-amphetamine treatment cessation, as the effects of continuous psychostimulant drug treatment can change following longer withdrawal periods [57, 63, 73]. The effects of different D-amphetamine doses on the endpoints assessed here also remain to be determined.

IntA produces successive "spikes" in both brain cocaine levels [26, 66] and horizontal locomotor activity, as seen here. Typically, there is a strong correlation between brain cocaine concentrations, extracellular DA concentrations in the striatum, and the time course of the locomotor activating effects of cocaine [74–76]. It is interesting, therefore, that D-amphetamine "flattened" the spikes in horizontal locomotor activity otherwise produced by intermittent cocaine intake (see also [30]). We speculate that D-amphetamine may have also attenuated the intermittent "spikes" in DA that would otherwise occur with IntA cocaine self-administration. Indeed, D-amphetamine maintenance treatment blunts the increase in NAc dopamine levels produced by acute cocaine injection [77]. The importance of intermittency in producing sensitization-related changes in brain and behavior has been long-recognised [49, 78–81], and therefore, D-amphetamine may have therapeutic effects because it blunts the intermittent spikes in striatal dopamine activity that promote the induction of sensitization. This hypothesis remains to be tested. In parallel, D-amphetamine reduced later cocaine seeking under extinction conditions. Rats are not exposed to cocaine during extinction tests, and so there may be more than one mechanism, besides blunting of sensitization-related changes in cocaine potency at the DAT, by which D-amphetamine reduces responding. Future research can determine the extent to which D-amphetamine modulates the development of dopamine- and glutamate-related plasticity linked to increased cocaine-seeking after abstinence.

Methodological considerations

During FSCV recordings, signal-to-noise ratio under cocaine-free conditions was sometimes suboptimal, and so we computed kinetic parameters as percentages relative to 0.3 µM cocaine. This has two main implications for the FSCV findings. First, the potential effects of D-amphetamine treatment on baseline DAT function or on baseline dopamine neurotransmission cannot be assessed. Therefore, it is not possible to account for any potential differences in baseline DAT function after cocaine self-administration/D-amphetamine treatment. However, the D-amphetamine treatment regimen used here does not significantly change basal dopamine uptake or dopamine D2-type autoreceptor activity in the NAc [25]. Second, any potential effects of D-amphetamine on the neurochemical actions of a low cocaine concentration (0.3 µM) also could not be determined. Relative to cocaine-free conditions, 0.3 µM can produce dopamine uptake inhibition, but the effect is quite small when comparing to the very large effects produced by higher cocaine concentrations ([25, 35, 82–84], albeit in some of these studies effects at 0.3 µM cocaine were shown without statistical comparisons to baseline). Moreover, prior work shows that D-amphetamine treatment does not change dopamine uptake inhibition produced by 0.3 µM cocaine in the NAc [25]. These previous findings notwithstanding [25], any potential group differences in either baseline dopamine uptake or in the magnitude of the effects of 0.3 µM cocaine at the DAT here could have influenced the effects observed at higher cocaine concentrations.

CONCLUSIONS

IntA cocaine self-administration models intermittent cocaine taking in humans [26, 29, 66], and was found to produce robust sensitization to both the horizontal locomotor-activating and DAT-inhibiting effects of cocaine in rats. This extends reports that IntA cocaine promotes sensitization to cocaine's dopamine-elevating

effects [31, 35, 36] (Reviewed in [22]). The original finding here is that D-amphetamine attenuates the cocaine-taking and -seeking behaviors otherwise promoted by IntA, and D-amphetamine may do this in part by blunting sensitization-related changes in cocaine potency at the DAT. This is consistent with the view that the transition to cocaine addiction involves sensitization-related neuroplasticity [71], and therefore, treatments that reverse this may be especially efficacious.

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AUTHOR CONTRIBUTIONS

FA designed the research, performed all behavioral experiments and analyzed all data with guidance from ANS. MPB contributed to rat behavioral testing in Experiment 1. BDL performed FSCV recordings with guidance from LET. VJ extracted apparent Km values from FSCV data. FA, TER, and ANS wrote the article with revisions from BDL, MPB, VJ, and LET.

REFERENCES

1. Vocci F, Ling W. Medications development: successes and challenges. Pharm Ther. 2005;108:94–108.
2. Fischer B, Blanken P, Da Silveira D, Gallassi A, Goldner EM, Rehm J, et al. Effectiveness of secondary prevention and treatment interventions for crack-cocaine abuse: a comprehensive narrative overview of English-language studies. Int J Drug Policy. 2015;26:352–63.
3. Sanchez-Hervas E. Cocaine addiction: treatments and future perspectives. Trends Psychiatry Psychother. 2016;38:242–3.
4. Negus SS, Henningfield J. Agonist Medications for the Treatment of Cocaine Use Disorder. Neuropsychopharmacology. 2015;40:1815–25.
5. Tardelli VS, Bisaga A, Arcadepani FB, Gerra G, Levin FR, Fidalgo TM. Prescription psychostimulants for the treatment of stimulant use disorder: a systematic review and meta-analysis. Psychopharmacology (Berl). 2020;237:2233–55.
6. Grabowski J, Rhoades H, Schmitz J, Stotts A, Daruzska LA, Creson D, et al. Dextroamphetamine for cocaine-dependence treatment: a double-blind randomized clinical trial. J Clin Psychopharmacol. 2001;21:522–6.
7. Greenwald MK, Lundahl LH, Steinmiller CL. Sustained release d-amphetamine reduces cocaine but not 'speedball'-seeking in buprenorphine-maintained volunteers: a test of dual-agonist pharmacotherapy for cocaine/heroin polydrug abusers. Neuropsychopharmacology. 2010;35:2624–37.
8. Rush CR, Stoops WW, Sevak RJ, Hays LR. Cocaine choice in humans during D-amphetamine maintenance. J Clin Psychopharmacol. 2010;30:152–9.
9. Shearer J, Wodak A, van Beek I, Mattick RP, Lewis J. Pilot randomized double blind placebo-controlled study of dexamphetamine for cocaine dependence. Addiction. 2003;98:1137–41.
10. Lile JA, Johnson AR, Banks ML, Hatton KW, Hays LR, Nicholson KL, et al. Pharmacological validation of a translational model of cocaine use disorder: Effects of d-amphetamine maintenance on choice between intravenous cocaine and a nondrug alternative in humans and rhesus monkeys. Exp Clin Psychopharmacol. 2020;28:169–80.
11. Czoty PW, Gould RW, Martelle JL, Nader MA. Prolonged attenuation of the reinforcing strength of cocaine by chronic d-amphetamine in rhesus monkeys. Neuropsychopharmacology. 2011;36:539–47.
12. Czoty PW, Martelle JL, Nader MA. Effects of chronic d-amphetamine administration on the reinforcing strength of cocaine in rhesus monkeys. Psychopharmacol (Berl). 2010;209:375–82.
13. Negus SS. Rapid assessment of choice between cocaine and food in rhesus monkeys: effects of environmental manipulations and treatment with d-amphetamine and flupenthixol. Neuropsychopharmacology. 2003;28:919–31.
14. Negus SS, Mello NK. Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a second-order schedule in rhesus monkeys. Drug Alcohol Depend. 2003;70:39–52.
15. Negus SS, Mello NK. Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a progressive-ratio schedule in rhesus monkeys. Psychopharmacol (Berl). 2003;167:324–32.
16. Chiodo KA, Lack CM, Roberts DC. Cocaine self-administration reinforced on a progressive ratio schedule decreases with continuous D-amphetamine treatment in rats. Psychopharmacol (Berl). 2008;200:465–73.
17. Chiodo KA, Roberts DC. Decreased reinforcing effects of cocaine following 2 weeks of continuous D-amphetamine treatment in rats. Psychopharmacol (Berl). 2009;206:447–56.
18. Thomsen M, Barrett AC, Negus SS, Caine SB. Cocaine versus food choice procedure in rats: environmental manipulations and effects of amphetamine. J Exp Anal Behav. 2013;99:211–33.
19. Zimmer BA, Chiodo KA, Roberts DC. Reduction of the reinforcing effectiveness of cocaine by continuous D-amphetamine treatment in rats: importance of active self-administration during treatment period. Psychopharmacol (Berl). 2014;231:949–54.
20. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science. 1987;237:1219–23.
21. Allain F, Minogianis EA, Roberts DC, Samaha AN. How fast and how often: The pharmacokinetics of drug use are decisive in addiction. Neurosci Biobehav Rev. 2015;56:166–79.
22. Kawa AB, Allain F, Robinson TE, Samaha AN. The transition to cocaine addiction: the importance of pharmacokinetics for preclinical models. Psychopharmacol (Berl). 2019;236:1145–57.
23. Lile JA. Pharmacological determinants of the reinforcing effects of psychostimulants: Relation to agonist substitution treatment. Exp Clin Psychopharmacol. 2006;14:20–33.
24. Samaha AN, Robinson TE. Why does the rapid delivery of drugs to the brain promote addiction? Trends Pharm Sci. 2005;26:82–7.
25. Siciliano CA, Saha K, Calipari ES, Fordahl SC, Chen R, Khoshbouei H, et al. Amphetamine reverses escalated cocaine intake via restoration of dopamine transporter conformation. J Neurosci. 2018;38:484–97.
26. Zimmer BA, Oleson EB, Roberts DC. The motivation to self-administer is increased after a history of spiking brain levels of cocaine. Neuropsychopharmacology. 2012;37:1901–10.
27. Algallal H, Allain F, Ndiaye NA, Samaha AN Sex differences in cocaine self-administration behaviour under long access versus intermittent access conditions. Addict Biol. 2019:e12809.
28. Allain F, Bouayad-Gervais K, Samaha AN. High and escalating levels of cocaine intake are dissociable from subsequent incentive motivation for the drug in rats. Psychopharmacol (Berl). 2018;235:317–28.
29. Beveridge TJR, Wray P, Brewer A, Shapiro B, Mahoney JJ, Newton TF Analyzing human cocaine use patterns to inform animal addiction model development. Published abstract for the College on Problems of Drug Dependence Annual Meeting, Palm Springs, CA. 2012.
30. Allain F, Samaha AN. Revisiting long-access versus short-access cocaine self-administration in rats: intermittent intake promotes addiction symptoms independent of session length. Addict Biol. 2018;24:641–51.
31. Calipari ES, Siciliano CA, Zimmer BA, Jones SR. Brief intermittent cocaine self-administration and abstinence sensitizes cocaine effects on the dopamine transporter and increases drug seeking. Neuropsychopharmacology. 2015;40:728–35.
32. James MH, Stopper CM, Zimmer BA, Koll NE, Bowrey HE, Aston-Jones G. Increased number and activity of a lateral subpopulation of hypothalamic orexin/hypocretin neurons underlies the expression of an addicted state in rats. Biol Psychiatry. 2019;85:925–35.
33. Kawa AB, Bentzley BS, Robinson TE. Less is more: prolonged intermittent access cocaine self-administration produces incentive-sensitization and addiction-like behavior. Psychopharmacol (Berl). 2016;233:3587–602.
34. Nicolas C, Russell TI, Pierce AF, Maldera S, Holley A, You ZB, et al. Incubation of cocaine craving after intermittent-access self-administration: sex differences and estrous cycle. Biol Psychiatry. 2019;85:915–24.

35. Calipari ES, Ferris MJ, Zimmer BA, Roberts DC, Jones SR. Temporal pattern of cocaine intake determines tolerance vs sensitization of cocaine effects at the dopamine transporter. Neuropsychopharmacology. 2013;38:2385–92.
36. Kawa AB, Valenta AC, Kennedy RT, Robinson TE. Incentive and dopamine sensitization produced by intermittent but not long access cocaine self-administration. Eur J Neurosci. 2019;50:2663–82.
37. Allain F, Roberts DC, Levesque D, Samaha AN. Intermittent intake of rapid cocaine injections promotes robust psychomotor sensitization, increased incentive motivation for the drug and mGlu2/3 receptor dysregulation. Neuropharmacology. 2017;117:227–37.
38. Richardson NR, Roberts DC. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods. 1996;66:1–11.
39. Wakabayashi KT, Weiss MJ, Pickup KN, Robinson TE. Rats markedly escalate their intake and show a persistent susceptibility to reinstatement only when cocaine is injected rapidly. J Neurosci. 2010;30:11346–55.
40. De Vries TJ, Schoffelmeer AN, Binnekade R, Mulder AH, Vanderschuren LJ. Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. Eur J Neurosci. 1998;10:3565–71.
41. Deroche-Gamonet V, Piat F, Le Moal M, Piazza PV. Influence of cue-conditioning on acquisition, maintenance and relapse of cocaine intravenous self-administration. Eur J Neurosci. 2002;15:1363–70.
42. Dong Y, Taylor JR, Wolf ME, Shaham Y. Circuit and Synaptic Plasticity Mechanisms of Drug Relapse. J Neurosci. 2017;37:10867.
43. Stewart J, de Wit H. Reinstatement of Drug-Taking Behavior as a Method of Assessing Incentive Motivational Properties of Drugs. In: Bozarth MA, editor. Methods of assessing the reinforcing properties of abused drugs. New York, NY: Springer New York; 1987. p. 211–27.
44. Fortin GM, Bourque MJ, Mendez JA, Leo D, Nordenankar K, Birgner C, et al. Glutamate corelease promotes growth and survival of midbrain dopamine neurons. J Neurosci. 2012;32:17477–91.
45. Sanchez G, Varaschin RK, Bueler H, Marcogliese PC, Park DS, Trudeau LE. Unaltered striatal dopamine release levels in young Parkin knockout, pink1 knockout, DJ-1 knockout and LRRK2 R1441G transgenic mice. PLoS ONE. 2014;9:e94826.
46. Varaschin RK, Osterstock G, Ducrot C, Leino S, Bourque MJ, Prado MAM, et al. Histamine H3 receptors decrease dopamine release in the ventral striatum by reducing the activity of striatal cholinergic interneurons. Neuroscience. 2018;376:188–203.
47. Davidson C, Lee TH, Ellinwood EH. Acute and chronic continuous methamphetamine have different long-term behavioral and neurochemical consequences. Neurochem Int. 2005;46:189–203.
48. Ellinwood EH, Davidson C, Yu GZ, King GR, Lee TH. Effect of daily dosing duration of direct and indirect dopamine receptor agonists: cocaine cross-tolerance following chronic regimens. Eur Neuropsychopharmacol. 2002;12:407–15.
49. Post RM. Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance. Life Sci. 1980;26:1275–82.
50. Sams-Dodd F. Effects of continuous D-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. Neuropsychopharmacology. 1998;19:18–25.
51. Kawa AB, Robinson TE. Sex differences in incentive-sensitization produced by intermittent access cocaine self-administration. Psychopharmacol (Berl). 2019;236:625–39.
52. Nicola SM, Deadwyler SA. Firing rate of nucleus accumbens neurons is dopamine-dependent and reflects the timing of cocaine-seeking behavior in rats on a progressive ratio schedule of reinforcement. J Neurosci. 2000;20:5526–37.
53. Oleson EB, Roberts DC. Behavioral economic assessment of price and cocaine consumption following self-administration histories that produce escalation of either final ratios or intake. Neuropsychopharmacology. 2009;34:796–804.
54. Liu Y, Roberts DC, Morgan D. Sensitization of the reinforcing effects of self-administered cocaine in rats: effects of dose and intravenous injection speed. Eur J Neurosci. 2005;22:195–200.
55. Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. Science. 1998;282:298–300.
56. Paterson NE, Markou A. Increased motivation for self-administered cocaine after escalated cocaine intake. Neuroreport. 2003;14:2229–32.
57. Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE. Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry. 2005;58:751–9.
58. Xue Y, Steketee JD, Sun W. Inactivation of the central nucleus of the amygdala reduces the effect of punishment on cocaine self-administration in rats. Eur J Neurosci. 2012;35:775–83.
59. Ferris MJ, Mateo Y, Roberts DC, Jones SR. Cocaine-insensitive dopamine transporters with intact substrate transport produced by self-administration. Biol Psychiatry. 2011;69:201–7.
60. Calipari ES, Ferris MJ, Jones SR. Extended access of cocaine self-administration results in tolerance to the dopamine-elevating and locomotor-stimulating effects of cocaine. J Neurochem. 2014;128:224–32.
61. Koob GF, Volkow ND. Neurobiology of addiction: a neurocircuitry analysis. Lancet Psychiatry. 2016;3:760–73.
62. Volkow ND, Koob GF, McLellan AT. Neurobiologic Advances from the Brain Disease Model of Addiction. N. Engl J Med. 2016;374:363–71.
63. Carr CC, Ferrario CR, Robinson TE. Intermittent access cocaine self-administration produces psychomotor sensitization: effects of withdrawal, sex and cross-sensitization. Psychopharmacology (Berl). 2020;237:1795–812.
64. Ahmed SH. The science of making drug-addicted animals. Neuroscience. 2012;211:107–25.
65. Edwards S, Koob GF. Escalation of drug self-administration as a hallmark of persistent addiction liability. Behav Pharm. 2013;24:356–62.
66. Zimmer BA, Dobrin CV, Roberts DC. Brain-cocaine concentrations determine the dose self-administered by rats on a novel behaviorally dependent dosing schedule. Neuropsychopharmacology. 2011;36:2741–9.
67. Ward AS, Haney M, Fischman MW, Foltin RW. Binge cocaine self-administration in humans: intravenous cocaine. Psychopharmacol (Berl). 1997;132:375–81.
68. Izenwasser S, Cox BM. Daily cocaine treatment produces a persistent reduction of [3H]dopamine uptake in vitro in rat nucleus accumbens but not in striatum. Brain Res. 1990;531:338–41.
69. Izenwasser S, Cox BM. Inhibition of dopamine uptake by cocaine and nicotine: tolerance to chronic treatments. Brain Res. 1992;573:119–25.
70. Challasivakanaka S, Zhen J, Smith ME, Reith MEA, Foster JD, Vaughan RA. Dopamine transporter phosphorylation site threonine 53 is stimulated by amphetamines and regulates dopamine transport, efflux, and cocaine analog binding. J Biol Chem. 2017;292:19066–75.
71. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev. 1993;18:247–91.
72. Willuhn I, Burgeno LM, Groblewski PA, Phillips PE. Excessive cocaine use results from decreased phasic dopamine signaling in the striatum. Nat Neurosci. 2014;17:704–9.
73. Dalia AD, Norman MK, Tabet MR, Schlueter KT, Tsibulsky VL, Norman AB. Transient amelioration of the sensitization of cocaine-induced behaviors in rats by the induction of tolerance. Brain Res. 1998;797:29–34.
74. Minogianis EA, Shams WM, Mabrouk OS, Wong JMT, Brake WG, Kennedy RT, et al. Varying the rate of intravenous cocaine infusion influences the temporal dynamics of both drug and dopamine concentrations in the striatum. Eur J Neurosci. 2019;50:2054–64.
75. Shou M, Ferrario CR, Schultz KN, Robinson TE, Kennedy RT. Monitoring dopamine in vivo by microdialysis sampling and on-line CE-laser-induced fluorescence. Anal Chem. 2006;78:6717–25.
76. Carey RJ, Damianopoulos EN, DePalma G. Differential temporal dynamics of serum and brain cocaine: relationship to behavioral, neuroendocrine and neurochemical cocaine induced responses. Life Sci. 1994;55:1711–6.
77. Johnson AR, Banks ML, Selley DE, Negus SS. Amphetamine maintenance differentially modulates effects of cocaine, methylenedioxypyrovalerone (MDPV), and methamphetamine on intracranial self-stimulation and nucleus accumbens dopamine in rats. Neuropsychopharmacology. 2018;43:1753–62.
78. Downs AW, Eddy NB. The effect of repeated doses of cocaine on the rat. J Pharmacol Exp Therapeutics. 1932;46:199–200.
79. Post RM, Rose H. Increasing effects of repetitive cocaine administration in the rat. Nature. 1976;260:731–2.
80. Reith ME, Benuck M, Lajtha A. Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. J Pharm Exp Ther. 1987;243:281–7.
81. Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. Behav Pharm. 1993;4:289–312.
82. Yorgason JT, Espana RA, Jones SR. Demon voltammetry and analysis software: analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. J Neurosci Methods. 2011;202:158–64.
83. Jones SR, Garris PA, Wightman RM. Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. J Pharm Exp Ther. 1995;274:396–403.
84. John CE, Jones SR. Voltammetric characterization of the effect of monoamine uptake inhibitors and releasers on dopamine and serotonin uptake in mouse caudate-putamen and substantia nigra slices. Neuropharmacology. 2007;52:1596–605.