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Are cocaine-seeking "habits" necessary for the development of addiction-like behavior in rats?

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Are cocaine-seeking "habits" necessary for the development of addiction-like behavior in rats?

Abbreviated Title: *Habit-formation and addiction-like behavior*

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There are no conflicts of interest to report

Abstract

Drug self-administration models of addiction typically require animals to make the same response (e.g., a lever-press or nose-poke) over and over to procure and take drugs. By their design, such procedures often produce behavior controlled by stimulus-response (S-R) habits. This has supported the notion of addiction as a “drug habit”, and has led to considerable advances in our understanding of the neurobiological basis of such behavior. However, for addicts to procure drugs, like cocaine, often requires considerable ingenuity and flexibility in seeking behavior, which, by definition, precludes the development of habits. To better model drug-seeking behavior in addicts we first developed a novel cocaine self-administration procedure (the Puzzle Self-Administration Procedure; PSAP) that required rats to solve a new puzzle every day to gain access to cocaine, which they then self-administered on an Intermittent Access (IntA) schedule. Such daily problem-solving precluded the development of S-R seeking habits. We then asked whether prolonged PSAP/IntA experience would nevertheless produce ‘symptoms of addiction’. It did, including escalation of intake, sensitized motivation for drug, continued drug use in the face of adverse consequences and very robust cue-induced reinstatement of drug-seeking, especially in a subset of ‘addiction-prone’ rats. Furthermore, drug-seeking behavior continued to require dopamine neurotransmission in the core of the nucleus accumbens (but not the dorsolateral striatum). We conclude that the development of S-R seeking habits is not necessary for the development of cocaine addiction-like behavior in rats.

Significance

Substance abuse disorders are often characterized as “habitual” behaviors aimed at obtaining and administering drugs. Although the actions involved in consuming drugs may involve a rigid repertoire of habitual behaviors, evidence suggests that addicts must be very creative and flexible when trying to procure drugs, and thus drug-seeking cannot be governed by habit alone. We modeled flexible drug-seeking behavior in rats by requiring animals to solve daily puzzles to gain access to cocaine. We find that habitual drug-seeking isn’t necessary for the development of addiction-like behavior, and that our procedure doesn’t result in transfer of dopaminergic control from the ventral to dorsal striatum. This approach may prove useful in studying changes in neuropsychological function that promote the transition to addiction.

Introduction

In defining "addiction", the Oxford English Dictionary (OED Online) cites an article from the Journal of the American Medical Association (1906), stating that, *"it matters little whether one speaks of the opium habit, the opium disease or the opium addiction"*. But is this correct? Is addiction equivalent to a "habit" (Tiffany, 1990; Everitt and Robbins, 2005, 2015; Lewis, 2015; Smith and Laiks, 2017)? In psychology, a habit refers to specific patterns of behavior controlled by stimulus-response (S-R) associations. Defining characteristics include automaticity, continued responding despite devaluation of the reward, as well as, "[increased] speed and efficiency, limited thought, rigidity, and integration of sequences of responses that can be executed as a unit" (Wood and Rünger, 2016; see also Graybiel, 2008). Certainly, behaviors involved in *consuming* drugs, once obtained, can be automated and habitual (Tiffany, 1990). But what about behaviors involved in *procuring* (seeking) drugs? In fact, to procure drugs, addicts typically show considerable ingenuity and flexibility in their behavior: first, to acquire the money to purchase drugs, then locate a possible drug source, and finally negotiate a purchase, often under very challenging circumstances (Preble et al., 1969; Neale, 2002; Heather, 2017). Such motivated, goal-directed behavior requires solving unique problems on a daily basis and, by definition, is not habitual.

However, animal self-administration studies of addiction often use procedures that necessarily promote both drug-seeking and -taking S-R habits (Vandaele and Janak, 2017). When animals are trained to make an action (e.g., a lever press) to receive an intravenous (IV) injection of a drug (and an associated cue), they quickly acquire self-administration behavior (Weeks, 1962). It is generally agreed that such behavior is initially controlled by learned associations between the act (lever press) and the outcome (IV drug; i.e., cognitive act-outcome [A-O] associations), as well as motivated by Pavlovian relationships between drug cues and drug effects that trigger incentive motivation (S-O associations; Everitt and Robbins, 2005). At this stage cocaine-seeking behavior is thought to be strongly controlled by dopamine activity in the ventral striatum (Robledo et al., 1992; Ito et al., 2004). However, with more prolonged drug experience there can be a gradual transfer of control over behavior from A-O (and S-O) associations to S-R habits, as behavior becomes more automatic and stereotyped, and this is accompanied increasing involvement of the dorsal (vs. ventral) striatum in the control of drug-seeking behavior (Ito et al., 2002; Di Ciano and Everitt, 2004; Vanderschuren et al., 2005; Belin and Everitt, 2008; Zapata et al., 2010). Thus, behaviors that are initially goal-directed and "shaped and maintained by [their] consequences" (Skinner, 1971), "increasingly become elicited as stimulus–response habits" (Everitt, 2014; see also Dickinson, 1985). In animal studies this

occurs in part because the *same* response must be repeated over and over to procure drug. In addition, the response is sometimes temporally separated from receipt of the reinforcer, as with interval schedules, which also promotes S-R habits (Dickinson, 1985; Dickinson et al., 1995; Everitt and Robbins, 2000; Wood and Rünger, 2016). However, unlike the act of drug-taking, the creativity and resourcefulness addicts must show to procure drugs suggests that this behavior is not dominated by habit (Preble et al., 1969; Neale, 2002; Heather, 2017).

Therefore, our aim was to first develop a cocaine self-administration procedure in rats that better reflects the flexible problem-solving required of addicts to procure drugs. To do this, like addicts, rats were required to solve a new problem every day to gain access to drug; simply repeating stereotyped actions that worked in the past would not suffice. This precluded the development of habitual drug-seeking behavior. Our second aim was to then use this procedure to ask whether S-R habits, and the associated transfer of behavioral control from the ventral to dorsal striatum, are indeed necessary for development of addiction-like behavior in rats, as assessed using behavioral economic indicators of cocaine demand (Zimmer et al., 2012; Bentzley et al., 2013; Kawa et al., 2016).

Materials and Methods

Subjects

Male Long-Evans rats (n=46, Charles River Laboratories), weighing 250-275 g on arrival, were individually housed in a temperature- and humidity-controlled vivarium on a reverse light cycle. After acclimating to housing conditions for one week with food and water available *ad libitum*, rats were held at a steady body weight (~90%; food restricted to ~ 25 g/day) for an additional week before experimental procedures commenced. Behavioral testing occurred during the dark phase of the light cycle. All procedures conducted according to a protocol approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Apparatus

Behavioral training took place in standard Med Associates operant chambers 22 × 18 × 13 cm) enclosed within ventilated sound-attenuating compartments. All manipulanda or conditioned stimulus (CS) devices were purchased from Med Associates. For all tests, a cue light was located on the center-top of the front side of the chamber, with a single retractable lever with a flat edge positioned below and either on the left or right side of the light. This lever will be referred to as the “taking” lever. Chambers were always equipped with a red house light on the back wall of the chamber, directly opposite the cue light. A speaker used for presentation

of a tone (see below) was positioned directly below the house light. The puzzle "seeking" manipulanda consisted of (1) a response wheel that made an audible click every quarter rotation; (2) a fixed lever with rolled edge; and (3) a nose port. These were positioned on the bottom-rear of the chamber (either to the left, right, or directly underneath the speaker). During initial training, a food cup was positioned on the front side of the chamber, below the cue light. Banana flavored pellets were delivered to this food cup via a dispenser mounted outside the chamber. Both the food cup and dispenser were removed during drug self-administration. For drug self-administration, responses on the retractable lever activated a syringe pump (mounted outside the sound-attenuating box), which delivered IV cocaine to the tethered rat via tubing connected to the rat's catheter back port.

Experimental Procedures

Food Training

The puzzles rats had to solve to gain access to a reward (food or drug; Figures 1, 2; Table 1) were very demanding and thus considerable training was required for them to acquire the task. For this reason, rats were initially trained to solve puzzles to gain access to a food reward, prior to catheter implantation. This was to better ensure that their catheters remained patent during the later prolonged cocaine self-administration phase of the experiment. Thus, rats were first familiarized with banana-flavored food pellets in their home cages for 2 days before experimental procedures began. Then, on a single pre-training day, rats were taught to retrieve the pellets from a food cup in the operant chambers according to a variable time 30-sec schedule (Figure 1, Stage 1). During the next two days, rats lever-pressed on the taking lever, which remained extended, to receive a total of 60 pellets/session on a fixed ratio 1 (FR1) schedule. Finally, rats began training on the "seeking" manipulanda (response wheel, rolled-edge lever, nose port), which were separately introduced during 3-day blocks. Each session began with the house light OFF and then turned ON after 60 seconds. The house light ON signaled that the "seeking" manipulanda were active (later referred to as "Puzzle-ON"). On the first day of each block, a single response on the respective seeking manipulandum resulted in a tone presentation (1 second), and subsequent extension of the taking lever. Rats were then allowed to lever-press for pellets (with 1-sec CS-light presentation) on an FR1 schedule for 1 minute. Then, the house light was turned off ("Puzzle-OFF"), and the taking lever retracted, signaling a 20-sec time-out period. The house light then turned back on, signaling the second trial (of 8 trials total) and enabling the rats to activate the seeking manipulandum. Similar procedures were used on the second and third days of each training block, but the number of

required responses on the seeking manipulandum was increased to 3. After completing the training block, the seeking manipulandum was removed and replaced with another one. These food training procedures were repeated until all rats learned the pattern of reward-seeking and –taking (completion of 8 trials during 2 consecutive days).

In a subset of rats (n=12; not used for cocaine self-administration), food training continued using puzzles similar to those described below in Table 1 (8 trials/day as described above, ~20 days total). Then, in counterbalanced order and separated by 3 additional days of puzzles, under extinction conditions reward-seeking was measured either after satiating the rats (rats were given 10 g of banana-flavored pellets before the test) or without satiating the rats.

Surgery

Following food training, rats were administered anesthesia (ketamine, 90 mg/kg, IP; xylazine, 10 mg/kg, IP) and underwent surgery for both 1) insertion of a catheter into the right jugular vein (as previously described, Crombag et al., 2000) and 2) implantation of bilateral guide cannulae aimed at either the NAc core (AP, +1.8; ML, ±1.6; DV, -5; mm from bregma and skull; Singer et al., 2016), or the DLS (AP, +1.2; ML, ±1.2; DV, -3; mm from bregma and skull; Vanderschuren et al., 2005). Guide cannulae were secured in place with surgical screws and dental acrylic. Both before surgery, and during recovery, rats were administered saline (5 ml, SC), the antibiotic cefazolin (100 mg/kg, SC), and the analgesic carprofen (5 mg/kg, SC). For the remainder of the experiment, IV catheters were flushed daily with sterile saline containing 5 mg/ml gentamicin sulfate to minimize infection and prevent occlusions. Rats were allowed to recover from surgery for 7 days before cocaine self-administration training began.

Infusion Criteria

The acquisition of drug self-administration took place over the course of 9 days, with only the taking lever present (Figure 1, Stage 2). During training, all rats were required to take the same amount cocaine hydrochloride (NIDA), as pre-determined by an infusion criteria (IC) procedure (Saunders and Robinson, 2010). Accordingly, rats gradually increased cocaine-taking from 10 to 40 infusions/day (IC10, 2 days; IC20, 3 days; IC40, 4 days; maximum 4 hrs/day). Each session started with a 1-min house light OFF period, followed by both the house light turning ON and extension of the taking lever (the same one used for food training). Rats were allowed to lever-press for cocaine on an FR1 schedule (0.4 mg/kg/infusion in 50 µl delivered over 2.6 s), and cocaine infusions were paired with the presentation of a cue light. The CS remained illuminated for 20 seconds, during which time subsequent lever presses had no

consequence. At the end of each session, after each rat completed the required number of infusions, the house light turned OFF and the rat was returned to its home cage. Rats that did not complete IC training within 9 days were excluded from the experiment (n=2).

Behavioral Economic Tests

After acquiring cocaine self-administration (n=34; 3 replications), baseline behavioral economic parameters were measured using a within-session threshold procedure, as described previously (Oleson and Roberts, 2009; Oleson et al., 2011; Bentzley et al., 2013; Kawa et al., 2016). Briefly, during five 110-min within-session threshold tests (one per day), rats were allowed to press the taking lever to receive cocaine. However, the dose of cocaine was decreased every 10 minutes according to a quarter logarithmic scale (383.5, 215.6, 121.3, 68.2, 38.3, 21.6, 12.1, 6.8, 3.8, 2.2, and 1.2 µg/infusion), without any timeout periods. During these tests, the cue light was presented during each drug infusion, while the house light was on for the entire session (except during the first 60 seconds). As described previously (Bentzley et al., 2013; Kawa et al., 2016), the drug-taking data were used to generate demand curves via a focused-fitting approach (typically utilizing the final 3 days of stable responding on the threshold procedure). Accordingly, for each rat, baseline measures were obtained for P_{max} (price of drug that elicited maximum responding), Q_O (preferred level of drug consumption when the price was negligible), and α (demand elasticity, normalized to Q_O).

Following the threshold procedure, rats were tested on a within-session punishment procedure for 3 days. As described previously (Bentzley et al., 2014; Kawa et al., 2016), during this test the dose of drug available for self-administration remained constant (38.3 µg/infusion), but the cost of drug gradually increased by imposing an adverse consequence for taking it (a footshock; 0.5 s). Briefly, after a 20-min period of cocaine administration (FR1) without punishment, the level of shock delivered concurrently with a drug infusion increased every 10 minutes (0.10, 0.13, 0.16, 0.20, 0.25, 0.32, 0.40, 0.50, 0.63, 0.79 mA). To normalize for individual variation, data were analyzed as the maximum current each rat was willing to endure to defend its preferred level of cocaine-intake.

Finally, after prolonged cocaine self-administration using an Intermittent Access procedure (IntA; see Figure 1, Stage 4), but before the saline- and cocaine-induced reinstatement tests, rats were once again tested on the within-session threshold (2 days) and punishment (2 days) behavioral economic procedures. This was to assess how cocaine demand *changed* from baseline, as a function of PSAP/IntA experience.

Puzzle Self-Administration Procedure with Intermittent Access to Cocaine

Following initial behavioral economic testing, rats self-administered cocaine for 4 weeks using a *Puzzle Self-Administration Procedure (PSAP)* specifically developed to maintain behavioral flexibility in drug-seeking behavior (Figure 1, Stage 3; Figure 2; 5 days/week; maximum 10 trials or 7 hours per session; average 9.41±0.095 completed trials across all sessions). Similar to standard Intermittent Access (IntA) self-administration protocols (Zimmer et al., 2012; Kawa et al., 2016), rats were allotted 5-min drug-available periods (FR1 on the extended taking lever; house light on), alternating with 25-min drug-unavailable time-out periods (taking lever retracted; house light off). When drug was available, each lever press resulted in a cocaine infusion (0.4 mg/kg/infusion in 50 µl of 0.9% sterile saline, delivered over 2.6 s; no post-infusion time-out) along with cue light presentation. However, in contrast to previous studies, rats needed to first complete a drug-seeking task on each trial (i.e., solve a puzzle; Table 1), before gaining access to the taking lever. During the first trial, and following each time-out period, puzzle availability ("Puzzle-ON"; and thus the initiation of drug-seeking) was signaled by the house light turning on. Since the puzzle manipulanda (response wheel, rolled-edge lever, nose port) were always present, some interaction did occur during "Puzzle-OFF" periods (e.g., time-outs), however, there was significantly more drug-seeking during "Puzzle-ON" than "Puzzle-Off" periods (comparison of drug-seeking rates, see results & Figure 4).

During each self-administration day, rats learned to solve a single unique puzzle to gain access to the taking lever. Across the entire experiment, puzzles were not repeated (except for "representative" puzzle #15, which was used during microinjection procedures described below). The order of puzzle testing was kept constant for all rats (Table 1). Also, puzzles gradually became more difficult as the experiment progressed, requiring an increasing number of drug-seeking responses (Puzzles/Days 1-3, 1 response required; Puzzles/Days 4-6, 2 resp. req.; Puzzles/Days 7-13, 3-5 resp. req.; Puzzles/Days 14-20, 5-6 resp. req.). Puzzle difficulty increased gradually because we found in pilot studies that the task was too difficult for the rats to master otherwise. Aside from Puzzles 1-3, which required only a single behavioral response for rats to gain access to the drug-taking lever, the remainder of the puzzles required rats to utilize 2 of the 3 manipulanda (2 series of responses). Successful completion of each response series resulted in the presentation of a tone (1 s). For example, Puzzle #15 (Figure 2) first required rats to press the rolled-edge lever 4 times in a row (essentially FR4), and this resulted in a tone presentation. This also signaled that responding on the rolled-edge lever was no longer required and that the rat must next respond on a different manipulandum (in this example, the wheel). Then, after 2 wheel turns, the tone would sound again, followed by

extension of the taking lever (beginning drug-available and “Puzzle-OFF period, while the house light remained on).

Importantly, however, during the “Puzzle-ON” period, mistakes on the puzzle resulted in the rat having to re-start the puzzle from the beginning. Thus, according to representative puzzle #15, extra presses on the rolled-lever (e.g., 5 presses instead of 4), or nose-poking instead of turning the wheel, would have “re-set” the puzzle from the beginning, again requiring 4 responses on the rolled-lever. Despite the difficult nature of the puzzles, rats did improve drug-seeking performance across trials during a given session (see results). Even so, to ensure that all rats got equal cocaine exposure across days, failure to solve the puzzle after a given period of time (trial 1, 10 minutes; trials 2-10, 15 minutes) resulted in the next drug-seeking response giving access to the taking lever, turning the puzzle off for that trial. Finally, because every rat differed in the amount of time taken to solve the puzzle, the amount of time between each drug-available period also differed (“Puzzle-ON” time + 25-min time-out), adding an extra-degree of drug intermittency when compared to other IntA experiments (Kawa et al., 2016).

Microinjections

The ability of DA signaling to regulate drug-seeking was assessed after 4 weeks of PSAP/IntA cocaine self-administration experience. Using a within-subject procedure, rats received microinjections of either vehicle or the DA receptor antagonist *cis*-(Z)-flupenthixol (0, 5, or 15 µg in 0.9% sterile saline; 0.5 µl/side/min, plus 1-min diffusion) into the NAc core (n=8) or the DLS (n=7), similar to previous reports (Di Ciano and Everitt, 2004; Vanderschuren et al., 2005; Murray et al., 2014). While rats were not divided according to addiction criteria for this analysis (described below), during prolonged PSAP/IntA self-administration on average all rats increased drug-seeking across sessions and there were no differences in drug-seeking between rats used in the DLS and NAc groups.

Microinjections were performed once every 3 days (doses counterbalanced, Latin-square design), with additional PSAP/IntA cocaine self-administration (novel puzzles, see Table 1) occurring on the 2 days separating the intracranial infusions. During microinjection test days, drug-seeking was tested on representative puzzle #15 (starting 5-minutes post-injection), allowing for easy comparison of behavior across doses. Also, on these test days, responding on the taking lever resulted in IV saline infusions, rather than cocaine, and PSAP/IntA testing was limited to ~3 hours. Because some rats stopped drug-seeking under these experimental conditions (flupenthixol, extinction), behavior was only analyzed for the first trial.

Cocaine-Induced Reinstatement

After completing the series of microinjections, rats were allowed to self-administer cocaine according to the PSAP/IntA schedule for an additional 2 days (novel puzzles). Then, following additional behavioral economic testing (Figure 1, Stage 4; 2 days threshold; 2 days punishment) and 2 more cocaine PSAP/IntA days (novel puzzles; followed by 2 days rest), rats were tested for cocaine-induced reinstatement of drug pursuit using procedures described previously (Deroche et al., 1999; Kawa et al., 2016). Briefly, tests were conducted over 2 days with the puzzle manipulanda removed. Each day began with the house light initially off (1-min) and then turned on for the remainder of the session. Next, on both sessions, the taking lever was extended, and rats underwent extinction for 90-min. After this period, in 30-min intervals rats received infusions of either IV saline (Day 1; 25, 50, 100, 200 µl) or cocaine (Day 2; 0.2, 0.4, 0.8, 1.6 mg/kg; same volume as corresponding saline injections).

Extinction and Cue-Induced Reinstatement

Rats underwent an extinction procedure (2 hours/session) for seven days after the cocaine reinstatement test. Consistent with other testing conditions, the house light was turned on 1-min after rats were placed in the operant chambers. During extinction, the drug-seeking manipulanda were removed, and the taking lever was extended throughout the session. Responses on the taking lever were without consequence. Next, the ability of the previously drug-paired cue light to reinstate pursuit of drug was tested, using a conditioned reinforcement procedure. Accordingly, rats were again tested under extinction, but each lever-press was reinforced with brief illumination of the cue light that had been previously paired with cocaine injections, along with activation of the infusion pump (2.6 s; no tubing attached).

Sacrifice and Histology

At the conclusion of the experiment, all rats were deeply anesthetized (sodium pentobarbital; 60 mg/kg, IP), and their brains were extracted and placed in formalin. Brains were later frozen, sliced using a cryostat (40 µm), and stained (cresyl violet) to confirm cannula tip placements within either the NAc core or DLS (Figure 8bc). Rats lacking correct bilateral cannula placements were not included in the analyses. Catheter patency was tested using brevital (0.1 ml, IV) after puzzles 20 and 26, as well as before sacrifice.

Experimental Design and Statistical Analysis

As described elsewhere, male Long-Evans rats (n=46) were trained on the various behavioral procedures. Microinjection procedures (injection site, dose) were counterbalanced according to principles of Latin-Square design. One-way or two-way repeated measures ANOVAs were used for analyzing all behavioral measures (Bonferroni corrections were used to control for multiple comparisons), except for responding during devaluation and extinction, for which paired or unpaired t-tests were used. All statistics were performed using GraphPad Prism.

Individual variation in addiction-like behavior was analyzed by determining whether rats met specific "addiction criteria", as described previously (Deroche-Gamonet et al., 2004; Kawa et al., 2016), and similar to criteria used to assess human substance abuse disorder in the DSM-5 (APA, 2013). First, we determined which rats displayed a) the greatest (top 1/3) motivation for drug (P_{max}), b) drug-taking despite adverse consequences (Max Charge endured), and c) greatest continued pursuit of drug despite it not being available (during extinction). Rats that met 2-3 of these benchmarks were classified as positively meeting addiction-like criteria (n=5), and the behaviors of these rats were compared to rats that met 0-1 addiction criteria (n=10). This distribution observed in Long Evans rats was similar to other strains, including Sprague Dawley rats (Kawa et al., 2016). Drug-seeking described in the current results was not used as a standard for determining 0-1 and 2-3 criteria rats because it was not included in previous reports (Deroche-Gamonet et al., 2004; Kawa et al., 2016). Some rats were not tested beyond the PSAP/IntA procedure or did not complete the entire experiment (i.e., through the cue-induced reinstatement test), and were thus excluded from the analyses of individual variation in motivation.

Importantly, the PSAP/IntA procedure is not meant to be a complete and all-encompassing animal model of addiction. For example, it is well-known that, when given the opportunity to obtain an “alternative reinforcer” to drug, animals and people will decrease their drug-use (Higgins, 1997; Venniro et al., 2017; for review, see Heather, 2017). This was not modeled in the present manuscript. We also did not incorporate measurements of impulsivity into the PSAP/IntA procedure (Dalley et al., 2011). Furthermore, like previous reports (e.g., Deroche-Gamonet et al., 2004; Kawa et al., 2016), we cautiously refer to the rats as displaying various “addiction-like” behaviors. While we and others believe that the behavioral economic and reinstatement techniques used have criterion validity (Epstein et al., 2006; MacKillop, 2016), the rats are not “addicts” and the complexity of human behavior obviously extends well-beyond what can be modeled in animals. That said, the lack of pre-clinical studies that have been translated into acceptable treatments for substance abuse may, in part, be due to incomplete or inadequate modeling of the human condition in animals. While it is, without a

doubt, difficult to mimic in rats the complex conduct of a “street addict” procuring drug, to the best of our knowledge PSAP/IntA is the first procedure that attempts to model this behavior in animals.

Results

Acquisition of Cocaine Self-Administration

Rats were first trained to lever-press for food and then to self-administer cocaine (data not shown). Rats readily increased responding for cocaine across training days (infusion criterion procedure; $F_{2,66}$=56.8, p<0.0001; one-way repeated measures ANOVA comparing lever-pressing across days; p<0.0001, taking lever responses on IC40 vs IC10 or IC20; p<0.05, IC20 vs IC10; Bonferroni). Similarly, rats spent more time self-administering drug when given the opportunity to take more cocaine ($F_{2,66}$=219.1, p<0.0001; one-way repeated measures ANOVA comparing session length across days; p<0.0001, IC40 vs IC10 or IC20, IC20 vs IC10, Bonferroni). Rats that did not administer 40 cocaine infusions on the final day of this procedure were excluded from further testing (n=2).

Puzzle Self-Administration Procedure with Intermittent Access to Cocaine

Drug-Seeking

After successfully learning to lever-press for cocaine, rats were allowed to self-administer cocaine for 20 days using the PSAP/IntA procedure (n=34). PSAP/IntA was designed to preclude the development of habitual drug-seeking across testing days. Accordingly, on each day rats needed to solve a single puzzle, for a total of 10 trials each day. It was possible, however, that rats were not learning to solve these puzzles, but were instead responding randomly on the drug-seeking manipulanda. To assess this possibility, we measured the rats’ within-session puzzle performance across days. Regardless of puzzle difficulty, rats improved their puzzle performance between the start and the end of testing each day (Figure 3a, Puzzles 4-6, $F_{2,66}$=4.11, p=0.02; Figure 3b, Puzzles 7-13, $F_{2,66}$=20.23, p<0.0001; Figure 3c, Puzzles 14-20, $F_{2,66}$=17.17, p<0.0001; one-way repeated measures ANOVAs), making a higher percentage of correct responses late in each session (trials 4-6 and/or 7-10; p<0.05-0.0001, Bonferroni) compared to earlier that day (trials 1-3). Despite this improvement, at the end of each session rats still only made correct responses ~45% of the time, indicating that the puzzles were quite difficult - rats continued to struggle to solve the puzzles each day, and more often than not they had to restart puzzles within a session. In addition, there was no improvement at the start of each session between days of the procedure. This indicates the

puzzles were sufficiently demanding to preclude the development of sterotyped, routine, or "habitual" behavior, but instead reflected motivated, goal-directed behavior throughout the PSAP/IntA schedule. This is consistent with increases in motivation to solve the puzzles to gain access to drug, with increasing puzzle and drug experience (see below).

Interestingly, it is possible that the rats' behavior during PSAP/IntA may have been governed by a series of semi-automated conscious sub-goals ruled by *if-then* conditions (implementation intentions; Sheeran, 2005; Wood and Rünger, 2016). This phenomenon has been referred to as a strategic automaticity and this differs from the unconscious automaticity commonly associated with habits (Gollwitzer and Schaal, 1998). In sum, it is not proficiency that is essential, but it is instead important that responding persists and must remain flexible as the rats make mistakes.

We next assessed how drug-seeking changed during prolonged PSAP/IntA cocaine self-administration. Because the difficulty of the puzzles increased as the experiment progressed (Table 1), drug-seeking was calculated as rate of responding (puzzle manipulanda activations normalized to the total amount of time needed to solve the puzzle; Puzzle-ON) and then compared to rate of responding during time-out periods (25-minute; Puzzle-OFF). Across the weeks of self-administration, rats significantly increased their rate of drug-seeking behavior (Figure 4a, Puzzle-ON black circles, Puzzle-OFF white circles; two-way repeated measures ANOVA comparing Puzzle-ON vs Puzzle-OFF responding across all trials; Effect of Session, $F_{3,99}=3.92$, $p=0.01$; Effect of Puzzle-ON/OFF, $F_{1,33}=35.06$, $p<0.0001$; Interaction between Session and Puzzle-ON/OFF, $F_{3,99}=3.36$, $p=0.02$; Puzzle-ON days 14-20 vs days 1-3 or 4-6, $p<0.0001$-0.01, Bonferroni). Drug-seeking was always greater during Puzzle-ON periods relative to Puzzle-OFF time-outs (Fig. 4a; $p<0.0001$-0.05, Bonferroni).

When rats made mistakes while trying to solve a puzzle, they were forced to restart the puzzle from the beginning (i.e., they had to again perform the first required behavioral response series; see Figure 2). Puzzles became harder to solve across sessions (see Table 1) and rats had difficulty solving later puzzles. Accordingly, the number occasions on which rats were forced to restart the puzzles increased across sessions (Figure 4b; $F_{3,99}=54.1$, $^*p<0.0001$). Importantly, despite this increase in failure rate, rats increased the rate at which they tried to solve the puzzles (Figure 4a), and they gradually got better at solving the puzzle during each session (Figure 3). The rats' perseverance in drug-seeking, and increased rate of responding, as the puzzles became progressively more difficult may reflect increasing motivation to procure drug, which is consistent with data from the behavioral economic measures of cocaine demand (see below). Furthermore, given they were required to constantly adjust their behavior, it would

be expected that drug-seeking would never become habitual, which is supported by further analyses below.

On a single test day, after 20 days of PSAP/IntA experience, the tones that normally signaled successful completion of each response chain were omitted, in a subset of rats. Note that these tones were neither paired with drug administration (they were not a drug CS) nor acted as a discriminative stimulus signaling drug availability. Indeed, more than 50% of the time a tone did not precede extension of the drug-taking lever, because more often than not the rats made a mistake after completing the first response chain, and had to restart the puzzle. Thus, the tones should not be interpreted as influencing behavior through properties of conditioned reinforcement, but instead they are “guide-tones” aiding in the performance of drug-seeking behavior. In contrast to the tones, the drug CS was the light cue paired with cocaine injections (and which was used in the test of reinstatement), and extension of the drug-taking lever was the discriminative cue that signaled drug availability. That said, omission of the “guide-tone” significantly decreased the rate of drug-seeking to the level seen during Puzzle-OFF periods (Figure 4a, cross-hatched square, subset of rats; t_5=2.61, p=0.048; paired t-test, days 14-20 vs. no tone responding). This indicates that these tones, which guided puzzle-performance but were not paired with drug-delivery, nevertheless powerfully motivated drug-seeking behavior. The nature of the psychological processes that allowed the tones to guide and motivate behavior are deserving of further investigation. Finally, because drug-seeking ceased in the absence of the tones, rats did not gain access to the taking-lever during this specific test session, and thus drug self-administration was not measured.

Lastly, in the drug-naïve subset of rats that were trained to seek and take sucrose pellets using a similar PSAP schedule (~20 days), devaluation of the reinforcer via satiation significantly decreased the pursuit of sucrose (reward-seeking puzzle responses, t_{11}=3.04, p=0.017; food receptacle entries, t_{11}=2.36, p=0.038; data not shown).

In summary, during PSAP/IntA: (1) Motivation to solve the puzzles increased, as indicated by an increase in rate of responding and response perseverance during the Puzzle-ON periods, even as puzzle difficulty increased (Fig. 4). (2) The rats never solved the puzzles on more than 35-45% of trials, and thus responding could never become automatized, as more often than not they had to restart the puzzle. (3) Rats could withhold responding when the puzzle was OFF and the guide-tones were absent (compare seeking when the puzzle was ON vs OFF; Fig. 4a). (4) The tones may have had motivational value that promoted continued drug-seeking, because their omission decreased seeking behavior to levels seen during Puzzle-OFF conditions (Fig. 4a). (5) The use of the PSAP procedure with a sucrose reward prevented the

development of SR-habits, as responding remained sensitive to devaluation of the reward. All of these data support the claim that drug-seeking never became "automatized" or habitual under PSAP/IntA conditions, and that seeking behavior remained sensitive to its consequences.

Drug-Taking

During the PSAP/IntA schedule, after rats correctly solved the puzzle on a given trial, they then gained access to the cocaine-taking lever for 5 minutes on an FR1 schedule, before a 25-min time-out period ensued. As shown in Figure 5a, on each trial, most cocaine infusions were taken during the first minute of the 5 minute period that rats had access to the drug, and escalation of cocaine-use occurred during this first minute of drug-availability across weeks of self-administration (Effect of Sessions 1-3 vs 14-20, $F_{1,33}$=35.46, p<0.0001; Effect of Trial, $F_{2,66}$=6.39, p=0.029; Session X Trial Interaction, $F_{2,66}$=8.25, p=0.0006; p<0.0001, any trial during days 1-3 vs any trial for days 14-20; Bonferroni). Furthermore, during early PSAP/IntA sessions (days 1-3), rats also increased their intake of cocaine across trials (during a session), taking more cocaine during trials 7-10 compared to either trials 1-3 or 4-6 (p<0.0001-0.01; comparing 1st minute of drug availability per trial; Bonferroni).

We did not directly assess whether drug-*taking* behavior became habitual. However, even after escalation of intake most drug-taking behavior consisted of taking 4-5 infusions in the first minute of drug availability and then stopping (presumably because brain levels of the drug rapidly reached Q_O; see below). It is hard to imagine that these 4-5 actions during each drug available period would transition from control by A-O associations to S-R associations, because the latter typically requires over-training. Furthermore, if drug-taking was completely habitual then we might have expected rats to continuously self-administer cocaine throughout the 5-minute drug-available period. Under this scenario, rats would have continued responding on the taking-lever even if they did not 'desire' or 'want' drug, similar to how overtraining rats to self-administer cocaine results in consistent drug-taking responses even if cocaine has been devalued (Miles et al., 2003). This, however, was not the case; rats took most of their cocaine infusions during the first minute of drug-availability. This restricted pattern drug-administration suggests that drug-taking, similar to drug-seeking, was not habitual. Nevertheless, we never attempted to devalue cocaine or otherwise test whether drug-*taking* came to be controlled by S-R associations, so we cannot address that issue here. That being said, rats did continue to show escalated cocaine intake beyond the first minute of drug-availability during late PSAP/IntA sessions (days 14-20; Effect of session across trials: 2nd min, $F_{1,33}$=6.23, p=0.02; 3rd min, $F_{1,33}$=5.78, p=0.02; 4th min, $F_{1,33}$=4.68, p=0.04; 5th min, $F_{1,33}$=3.96, p=0.05).

Rats also escalated their total daily cocaine-intake across the weeks of PSAP/IntA self-administration ($F_{3,99}$=4.94, p=0.0031, one-way repeated-measures ANOVA; data not shown), responding more on the taking lever during later sessions (days 14-20) compared to earlier sessions (days 1-3 or 4-6; p<0.01-0.05, Bonferroni). This escalation of cocaine taking was particularly evident during the first daily trial (Figure 5b; $F_{3,99}$=11.44, p<0.0001, one-way repeated-measures ANOVA of Infusions; p<0.0001-0.05, days 14-20 vs. 1-3 or 4-6; p<0.01, days 7-13 vs. 1-3; Bonferroni). The sensitization of these responses, both within- and across-sessions, suggests that with prolonged PSAP/IntA experience the rats developed one feature of addiction-like behavior, escalation of intake, consistent with previous reports (Kawa et al., 2016; Allain et al., 2017; Pitchers et al., 2017).

Tests for Addiction-Like Behavior

A major goal of this study was to develop an animal model of substance abuse disorder that better reflects the flexible drug-seeking behavior that typically characterizes the behavior of drug users as they transition to addiction. When modeling addiction-like behavior in animals, it is important to consider that not everyone who experiments with drugs goes on to compulsively abuse drugs. Furthermore, the DSM-5 attempts to quantify the severity of Substance Use Disorders by determining the number of symptoms individuals suffer from. To model this individual variation in animals, we first identified rats meeting either the most (2-3 criteria rats; n=5) or fewest (0-1 criteria rats; n=10) criteria of addiction, as previously described by Deroche-Gamonet et al., (2004), and in our recent paper using the IntA procedure (Kawa et al., 2016; also see the Data Analysis section of the present manuscript). Of course, animals in the top third on a measure used as an addiction “criteria” will score high on that measure after PSAP/IntA. The relevant question for this analysis concerns the extent to which motivation for cocaine *changed* in 0-1 vs 2-3 criteria rats. That is, did these subgroups always differ on measures of cocaine demand, or, were they similar before PSAP/IntA experience but come to differ only as a result of PSAP/IntA experience – did the experience change them differently. The results indicate the latter.

Individual Variation in Seeking and Taking Cocaine

During the initial acquisition of cocaine self-administration (IC procedure), there were no differences between 0-1 and 2-3 criteria rats in lever-presses made (Figure 6a; Effect of Group, $F_{1,13}$=0.061, p=0.81; Effect of IC, $F_{2,26}$=50.92, p<0.0001; Group X IC Interaction, $F_{2,26}$=0.36, p=0.70), and in fact, the 2-3 criteria rats were on average slower to obtain 20 or 40 infusions

(Figure 6b; Effect of Group, $F_{1,13}$=17.78, p=0.001; Effect of IC, $F_{2,26}$=122.00, p<0.0001; Group X IC Interaction, $F_{2,26}$=3.81, p=0.035; Bonferroni post-hoc tests, 0-1 vs 2-3 criteria rats for IC 20 or 40, p<0.001-0.01). Next, we re-analyzed the PSAP/IntA self-administration data as a function of addiction criteria. The 0-1 and 2-3 criteria rats did not differ in their rate of drug-seeking behavior prior to IntA experience (responses/min while solving puzzles), but with prolonged PSAP/IntA experience the rate of drug-seeking significantly increased in 2-3 criteria rats, but not 0-1 criteria rats (Figure 6c, Puzzle-ON; Effect of Session, $F_{1,13}$=15.22, p=0.0018; Effect of Group, $F_{1,13}$=1.09, p=0.32; Session X Group Interaction, $F_{1,13}$=10.43, p=0.0066; PSAP/IntA days 1-3 vs 14-20, p<0.01 for 2-3 criteria rats; 0-1 vs 2-3 criteria rats, p<0.05 during PSAP/IntA days 14-20). In contrast, there were no differences between 0-1 and 2-3 criteria rats in drug-seeking during the 25-min timeout periods, suggesting that all rats readily discriminated between drug-available and –unavailable periods (data not shown; PSAP/IntA days 1-3 vs 14-20; Effect of Group, $F_{1,13}$=0.24, p=0.63; Effect of Session, $F_{1,13}$=2.45, p=0.14; Group X Session Interaction, $F_{1,13}$=1.97, p=0.18).

Regarding the number of cocaine infusions taken across days of the PSAP/IntA procedure, there was a significant effect of early vs. late sessions (Figure 6d; Effect of Session, $F_{1,13}$=17.89, p<0.0010; Effect of Group, $F_{1,13}$=0.081, p=0.78; Session X Group Interaction, $F_{1,13}$=2.53, p=0.14). The 2-3 and 0-1 criteria rats did not differ in drug intake early, but by the end of PSAP/IntA, the 2-3 criteria rats significantly escalated their cocaine-intake (p<0.01, sessions 1-3 vs 14-20), while 0-1 criteria rats did not, although total intake did not differ significantly. Therefore, during late PSAP/IntA sessions (days 14-20), all rats took approximately the same amount of cocaine. It seems that while rats differed in motivation to seek cocaine, in the end, they did not differ in the amount of drug they preferred to take when it was available. Supporting this idea, regardless of the addiction-criteria group, PSAP/IntA experience did not significantly change the rats' preferred level of drug consumption when the price was negligible (Q_O; Figure 7c; Effect of Baseline (BL) vs. Post PSAP/IntA Tests, $F_{1,13}$=1.74, p=0.21; Effect of Group, $F_{1,13}$=0.39, p=0.54; BL/Post Test X Group Interaction, $F_{1,13}$=0.00024, p=0.99; calculations derived from the behavioral economic "threshold" procedure). Together, these results suggest that while individual variation exists in motivation to seek cocaine after PSAP/IntA experience, the preferred brain concentration of cocaine, which is what is defended when cost increases and is measured by Q_O, did not differ between the groups, and did not change with increasing drug experience. There appears to be a dissociation, therefore, between whatever desired drug effects determine Q_O, and the degree to which rats are motivated to obtain such effects, as we have reported previously (Kawa et al., 2016).

Behavioral Economic Assessment of Changes in Cocaine Demand as a Function of PSAP/IntA Experience

Cocaine demand was assessed both before (baseline, BL) and after (post-test) prolonged PSAP/IntA self-administration experience. During the "threshold" test the cost of cocaine was progressively increased by increasing the number of lever presses required to maintain the preferred brain level of cocaine. One measure of motivation for cocaine is the point at which the "cost of drug" was so high that rats were unwilling to continue "paying" (responding) (P_{max}; Figure 7a). Prior to PSAP/IntA the 0-1 and 2-3 criteria groups did not differ in P_{max}, and PSAP/IntA resulted in a significant increase (sensitization) in P_{max} in both groups, but the increase in P_{max} was signfificantly greater in 2-3 than 0-1 criteria rats (Effect of BL vs. Post PSAP/IntA Tests, $F_{1,13}$=27.57, p=0.0002; Effect of Group, $F_{1,13}$=7.63, p=0.016; BL/Post Test X Group Interaction, $F_{1,13}$=9.62, p=0.0084;p<0.001, Bonferroni). Also, after weeks of the PSAP/IntA procedure the demand curves became more inelastic in all rats, and the two groups did not differ on this measure (Figure 7b; α; Effect of BL vs. Post PSAP/IntA Test, $F_{1,13}$=10.50, p=0.0064; Effect of Group, $F_{1,13}$=0.79, p=0.39; BL/Post Test X Group Interaction, $F_{1,13}$=0.00069, p=0.98). Together, these findings suggest that prolonged cocaine self-administration using the PSAP/IntA procedure resulted in sensitized motivation for cocaine (increased P_{max} & decreased α), but no change in the preferred brain concentration of cocaine (Q_O).

People with a substance use disorder often continue taking drug in the face of enduring negative consequences. To model this, we asked whether or not rats would continue self-administering cocaine despite receiving increasing amounts of foot shock. There was no difference in the Max Charge 0-1 and 2-3 criteria rats were willing to endure in order to take cocaine prior to PSAP/IntA experience. However, with prolonged cocaine experience, there was a significant increase Max Charge in the 2-3 (but not 0-1) criteria rats (Figure 7d; BL/Post Test X Group Interaction, $F_{1,13}$=7.35, p=0.018; Effect of BL vs. Post PSAP/IntA Test, $F_{1,13}$=0.29, p=0.60; Effect of Group, $F_{1,13}$=1.50, p=0.24; p<0.05, 0-1 vs. 2-3 criteria rats during post-PSAP/IntA test, Bonferroni). Similar findings have been reported elsewhere (Deroche-Gamonet et al., 2004), where only a small proportion of animals developed compulsive drug-use despite negative consequences.

Individual Variation in Cocaine- and Cue-Induced Reinstatement

Even for people who are addicted, but have been able to stop, re-exposure to either their drug of choice, or to drug-associated cues, can instigate relapse into drug abuse (e.g.,

615 Anggadiredja et al., 2004). This long-lasting aspect of addiction can be modeled in rats by
616 measuring how a cocaine-priming injection, or exposure to a previously drug-paired CS, can
617 reinstate the pursuit of drug. In the present study, the reinstatement of drug-pursuit was
618 measured after prolonged PSAP/IntA cocaine self-administration (see Figure 1 timeline). First,
619 during a single extinction session, rats meeting 2-3 addiction-criteria responded more on the
620 lever that was previously used to take drug, compared to the 0-1 criteria rats (Figure 7e;
621 t_{13}=2.72, p=0.018). The next day, non-contingent IV cocaine infusions were administered and
622 these dose-dependently increased responding on the taking lever, regardless of whether or not
623 rats met "criteria for addiction" (Figure 7f; Effect of Drug Dose, $F_{4,52}$=4.01, p=0.0065; Effect of
624 Group, $F_{1,13}$=2.07, p=0.17; Dose X Group Interaction, $F_{4,52}$=0.29, p=0.88). Thus, after being re-
625 exposed to drug, all rats were liable to "relapse" into drug-pursuit, regardless of the number of
626 "addiction-criteria" they met.

627 After the drug-reinstatement test, rats underwent 7 daily extinction sessions followed by
628 a test for cue-induced reinstatement (conditioned reinforcement; CR). Similar to above, on the
629 first (Ext1) and second (Ext2) days of extinction the 2-3 criteria rats responded more on the
630 lever that was previously used to take drug (Figure 7g; Effect of Group, $F_{1,13}$=32.75, p<0.0001;
631 Effect of Session, $F_{6,78}$=2.53; Effect of Group vs. Session, $F_{6,78}$=1.80, p=0.11; 0-1 vs 2-3 crit. rats
632 for Ext1 or Ext2, p<0.001, Bonferroni), but this group difference was no longer evident after 7
633 days of extinction (Ext7). Drug-seeking was not assessed following extinction and is thus worthy
634 of future investigation.

635 Next, the cocaine-associated light CS reinstated responding on the taking lever (under
636 extinction conditions) significantly in both groups (Figure 7h; Effect of Group, $F_{1,13}$=14.29,
637 p=0.0023; Effect of Ext7 vs. CR Session, $F_{1,13}$=36.44, p<0.0001; p<0.001-0.05, Ext7 vs CR for
638 either 0-1 or 2-3 crit. rats, Bonferroni), but this effect was more robust in 2-3 criteria rats relative
639 to rats meeting 0-1 addiction-criteria, as indicated by a significant interaction effect (Group X
640 Ext7/CR Session Interaction, $F_{1,13}$=8.72, p=0.011; p<0.001, 0-1 vs. 2-3 crit. rats on CR test,
641 Bonferroni). This effect was evident both during the first and second hours of the test (Effect of
642 Group, $F_{1,13}$=11.90, p=0.0043; Effect of Time, $F_{1,13}$=0.76, p=0.40; Group X Time Interaction,
643 $F_{1,13}$=0.085, p=0.78; p<0.01-0.05, 2-3 vs. 0-1 crit. rats at either time-point, Bonferroni). Thus,
644 following PSAP/IntA experience, re-exposure to cocaine reinstated similar pursuit of drug in all
645 rats, whereas re-exposure to drug-related conditioned stimuli reinstated greater pursuit of drug
646 in rats characterized as being most "addiction-prone." The different propensities across rats for
647 drug- and cue-induced reinstatement suggests a dissociation between their neurobehavioral
648 underpinnings (Epstein et al., 2006). Accordingly, some psychopharmacologic therapies may be

ideal for preventing cue-induced relapse to a greater extent than drug-induced relapse (Anggadiredja et al., 2004).

Drug-Seeking & DA Neurotransmission

DA neurotransmission within the ventral striatum (NAc core) is believed to mediate motivated goal-directed drug-seeking (i.e., not habitual), while DA signaling within the DLS is thought to underlie habitual drug-seeking (i.e., not goal-directed; Everitt, 2014). Given that the PSAP/IntA procedure models prolonged non-habitual drug-seeking behavior, we predicted that blocking DA signaling in the NAc core, but not in the DLS, would decrease drug-seeking behavior. To test this, after weeks of PSAP/IntA self-administration, we measured drug-seeking after microinjecting the DA receptor antagonist flupenthixol (0, 5, or 15 µg) into either the NAc core or DLS. The effect of flupenthixol on drug-seeking was dependent upon which dose was injected into what brain region (Figure 8a; Brain Region X Drug Dose Interaction, $F_{2,26}$=8.30, $p<0.0016$; Brain Region, $F_{1,13}$=3.99, p=0.067; Effect of Drug Dose, $F_{2,26}$=2.47, p=0.10; two-way repeated measures ANOVA; individual variation not measured due to sample size). When injected into the NAc core, both doses of flupenthixol reduced drug-seeking relative to vehicle ($p<0.05$, Bonferroni). In contrast, when injected into the DLS, the lower dose of flupenthixol enhanced drug-seeking (5 µg; $p<0.05$, vs. DLS veh or 15 µg; $p<0.01$, vs. NAc 5 µg), but the higher dose of flupenthixol (15 µg) had no effect.

The surprising finding that the low dose of flupenthixol into the DLS actually increased drug-seeking may be consistent with the idea that the ventral and dorsal striatum interact to regulate drug-seeking. Perhaps the DLS serves as a “brake” on aberrant ventral striatal activity and motivational processes. In fact, it has recently been proposed that suppression of the ventral striatum by the DLS may help limit reward-seeking to specific contexts in which reward is likely to be available (via processes of conditioned inhibition, although the exact mechanism remains unclear; Schneck and Vezina, 2012). Thus, it could be hypothesized that blockade of DA signaling in the DLS disinhibited drug-seeking (as seen following 5 µg flupenthixol), both in the normal cocaine self-administration environment, as well as in locations where the rat had never before experienced drug. Accordingly, this could result in decreased efficiency in seeking and procuring drug (Willuhn et al., 2012).

Together, these findings suggest that, even after prolonged cocaine self-administration under PSAP/IntA conditions, DA in the NAc core retains control over drug-seeking behavior. Furthermore, the surprising observation of enhanced drug-seeking following DA blockade in the DLS may suggest a novel role for this brain region in the regulation of motivated behavior.

683
684 **Discussion**

685 Each day addicts are typically faced with unique and constantly changing circumstances,
686 and procuring drugs often requires considerable ingenuity and problem-solving, conditions not
687 conducive to the development of habits (Gillan et al., 2015; Halbout et al., 2016; Heather, 2017).
688 As put by Tiffany (1990), “A street addict who daily must find a new way of obtaining heroin
689 would never be able to fully automatize those components of his or her drug-use behavior”.
690 Indeed, such individuals have been described as “economic entrepreneurs” (Preble et al., 1969)
691 who must constantly be “taking care of business” (see also Neale, 2002; Heather, 2017). To
692 model such flexible patterns of drug-seeking in rats, a cocaine self-administration procedure
693 (PSAP) was developed that required rats to solve a new problem (puzzle) each day to gain
694 access to cocaine, which was then taken on an Intermittent Access (IntA) schedule (Zimmer et
695 al., 2012; Kawa et al., 2016). This procedure precluded S-R seeking habits, but nevertheless,
696 produced addiction-like behavior, especially in susceptible rats. Furthermore, cocaine-seeking
697 was reduced by DA antagonism in the NAc core, but not the DLS. We conclude that neither S-R
698 habits, nor a transfer of behavioral control from the ventral to the dorsal striatum, are necessary
699 for the development of addiction-like behavior in rats.
700
701 Puzzle Self-Administration Procedure

702 What is the evidence that drug-seeking behavior during PSAP/IntA was not controlled by
703 S-R habits? Presenting this work we have heard the comment that maybe the rats “get into the
704 habit” of solving puzzles. This comment underscores the importance of differentiating between
705 colloquial use of the word “habit”, and its scientific definition. In psychology, habits refer to
706 stereotyped, automatic, rigid and relatively inflexible behaviors, that through over-training come
707 to be evoked by specific stimuli (S-R), largely independent of the value of the goal (Dickinson,
708 1985; Dickinson et al., 1995; Graybiel, 2008; Everitt, 2014; Gasbarri et al., 2014; Wood and
709 Rünger, 2016). That does not characterize cocaine-seeking behavior in the present study. For
710 example, seeking behavior decreased dramatically when the tone that signaled completion of
711 each response component of the daily puzzle was omitted, indicating it remained sensitive to its
712 consequences. Also, in rats trained to seek and take sucrose using the PSAP, devaluation of
713 the reward decreased responding. Furthermore, during PSAP/IntA the rats’ never made more
714 than ~45% correct responses, so they frequently had to restart a given puzzle. Both within and
715 between sessions they had to struggle to solve the daily puzzle necessary to get access to

716 cocaine, and they became increasingly motivated to do so. Therefore, the puzzles were
717 sufficiently demanding that seeking behavior could never become “automatized”.
718
719 Tests for Addiction-Like Behavior
720 What is the evidence that the rats developed addiction-like behavior? As in other studies
721 on this topic (Deroche-Gamonet et al., 2004; Belin and Everitt, 2008), we asked whether drug
722 experience produced symptoms that are diagnostic of substance use disorders (APA DSM-5,
723 2013). The development of addiction-like behavior was indicated by: (1) an increase in how
724 avidly cocaine was sought (seeking responses/min); (2) escalation of intake; (3) a greater
725 willingness to defend the preferred level of consumption as cost increased, in either effort
726 required (increased P_{max} and decreased α) or (4) upon the imposition of an adverse
727 consequence (Max Charge); (5) resistance to extinction; and (6) very robust cue-induced
728 “relapse”. We suggest these effects were likely due to enhanced incentive motivation (incentive-
729 sensitization), because when cocaine had negligible cost, consumption was unchanged (Q_O;
730 see also Kawa et al., 2016). Although highly speculative, this is suggestive of increased
731 “wanting”, but not “liking (Robinson and Berridge, 1993).
732 However, there is considerable individual variation in susceptibility to addiction, and
733 most people who try cocaine do not go on to develop addiction (Anthony et al., 1994). There
734 was also considerable individual variation in addiction-like behavior in the present study.
735 Although PSAP/IntA experience increased motivation for drug in most rats, on some measures
736 it was especially effective in doing so in rats identified as “addiction-prone” (2-3 criteria rats). It is
737 critical to note that 0-1 and 2-3 criteria rats did not differ *prior* to PSAP/IntA experience, but this
738 experience produced more robust incentive-sensitization in 2-3 criteria rats.
739 PSAP was coupled to the recently developed IntA self-administration procedure to better
740 mimic patterns of cocaine-taking in humans, especially during the transition to addiction, when
741 the pattern of cocaine use is very intermittent, both between and within bouts of use (Beveridge
742 et al., 2012; Zimmer et al., 2012; Allain et al., 2015; Kawa et al., 2016). Under IntA conditions
743 rats take much less cocaine than with more common long-access (LgA) procedures, in which
744 rats have continuous access for at least 6 hours (Ahmed and Koob, 1999; Zimmer et al., 2012).
745 Despite taking much less drug, IntA produces a greater increase in motivation for cocaine than
746 LgA (Zimmer et al., 2012; Kawa et al., 2016). Furthermore, IntA produces psychomotor
747 sensitization, and the degree of psychomotor sensitization predicts the magnitude of the
748 increase in motivation for drug (Allain et al., 2017), and also results in sensitized DA
749 neurotransmission (Calipari et al., 2014). Finally, the magnitude of cue-induced reinstatement

seen here (~150 responses/hour) and by Kawa et al. (2016), was much greater than typically seen with either short- or LgA procedures (60-80 responses/hour; Grimm et al., 2003; Saunders and Robinson, 2010). These findings suggest that the temporal pattern of cocaine use importantly influences the development of addiction-like behavior (Allain et al., 2015), even in the absence of S-R habits.

Drug-Seeking & DA Neurotransmission

It is often argued that, with prolonged drug self-administration, regulation over drug-seeking shifts from being controlled by DA transmission in the NAc, to DA signaling in the DLS (Ito et al., 2002; Di Ciano and Everitt, 2004; Vanderschuren et al., 2005; Belin and Everitt, 2008; Zapata et al., 2010). Based on this functional neuroanatomy, S-R habit hypotheses of addiction suggest that drug-seeking transitions from being regulated by A-O associations and S-O motivational processes, to being dictated by S-R habits (Everitt, 2014). Given we found that drug-seeking habits are not necessary for the development of addiction-like behavior, we asked whether DA neurotransmission in the NAc and/or DLS regulate drug-seeking following PSAP/IntA. The inhibition of DA receptors in the NAc, using the DA receptor antagonist flupenthixol, reduced drug-seeking (at both doses tested). In contrast, inhibition of DA receptors in the DLS either enhanced (low dose) or had no effect (high dose) on drug-seeking. This suggests that the development of addiction-like behavior may not require a transfer of dopamine control from the ventral to the dorsal striatum.

Other evidence suggests that linking the DLS only to S-R habits may be over-simplistic. Elegant experiments disconnecting the unilateral NAc core from the contralateral DLS suggest that communication between these regions is necessary for drug-seeking (Belin and Everitt, 2008). Others have shown that the DLS regulates motivated responding to cues (DiFeliceantonio and Berridge, 2016) and action-outcome assocations (Burton et al., 2017). Also, lesions of either the ventral or dorsal striatum reduce motivated responding for cocaine on a progressive ratio schedule (Suto et al., 2011). Furthermore, across short access cocaine self-administration sessions (ShA; 3-wks, 1 hr/d) DA transmission shifts from the NAc to the DLS in the absence of drug-seeking habits (Willuhn et al., 2012) and, surprisingly, there is no such shift in DA signaling when rats are trained using LgA procedures (despite escalating drug-intake; Willuhn et al., 2014). In contrast, imaging studies of substance abusers demonstrate greater DA signaling in the dorsal striatum than in the NAc when they are presented with drug-cues (Volkow et al., 2006; Vollstädt-Klein et al., 2010; Jasinska et al., 2014; but also see evidence for release in the NAc - Boileau et al., 2007; Leyton and Vezina, 2012). While this has been characterized

as the “activation of DA pathways that trigger the behavioral habits leading to compulsive drug seeking and consumption” (Volkow et al., 2006), cues were presented non-contingently and not during the performance of a S-R habit. Therefore, it’s difficult to say if the dorsal striatal activations observed in cocaine addicts reflect habitual or incentive motivational processes.

Conclusion

Cocaine self-administration using PSAP coupled with IntA, which precluded the development of S-R drug-seeking habits, nevertheless resulted in the emergence of addiction-like behavior, especially in susceptible rats. Furthermore, under these conditions cocaine-seeking required intact DA neurotransmission in the core of the NAc, but not in the DLS. The nature of the psychological and neural processes that control behavior are very dependent on the conditions under which behavior is studied, and some drug self-administration procedures may be useful for studying the automated habits that sometimes characterize drug consumption. However, the procedures described here may better model patterns of drug-seeking and -taking behavior as drug users transition to addiction, and thus, may be especially useful in determining what changes in what neuropsychological processes lead to this transition.

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Tables

Table 1: PSAP Schedule of Puzzles

All puzzles used during PSAP are shown. The first 20 puzzles were used during the initial PSAP/IntA procedure. Puzzles 21-28 were used for 2-day blocks between tests of drug-seeking and motivation for drug (see Figure 1). A single puzzle was tested each day, with 10 trials per day (or after 7 hours had elapsed).

Figures

Figure 1: Schedule of Experimental Procedures

The experimental procedures are divided into four stages: (1) food training (data not shown), (2) cocaine self-administration training, (3) the PSAP/IntA procedure and drug-seeking tests, and (4) final tests of addiction-like behavior. See Table 1 for a description of PSAP puzzles 1-28.

Figure 2: Diagrammatic Representation of the Puzzle Self-Administration (Seeking) and Intermittent Access Cocaine–Taking Procedure (PSAP/IntA)

The behavior required to solve Puzzle #15 is illustrated. The drug-seeking phase requires the completion of 2 distinct response sequences. In this example, the 1st response series requires the rat to make 4 presses on the rolled-edge lever. If successful (correct responses denoted by solid/thick lines), this is followed by a 1-sec tone, and then the rat must complete the 2nd response series, consisting here of 2 wheel turns. If this is also successful, the tone sounds again and this is followed by insertion of the taking lever and the transition to the drug-taking phase. However, if either the 1st or 2nd response sequence during the drug-seeking phase is performed incorrectly (indicated by dashed lines), no tone is presented and the animal would have to reinitiate the 1st response series (i.e., restart the puzzle from the beginning). For example, for this puzzle, if a rat initially responded on either the nose poke hole or wheel they would not hear any tone, until they figured out 4 responses on the rolled lever were required. Furthermore, if, after 4 responses on the rolled lever resulted in a tone, they next respond on either the nose poke or made another response on the rolled lever, then the puzzle would reset. However, after successful completion of the second response series the taking lever would extend into the chamber and the rat is allowed to self-administer cocaine on an FR1 schedule, with no timeout, for 5 min. Each cocaine infusion is presented along with a CS light. After 5

minutes the drug-taking lever retracts, the houselight is turned off, and a 25 min timeout period begins. After the 25 min timeout period, the houselight is turned back on and another trial of PSAP/IntA is initiated (10 trials or 7 hours/day).

Figure 3: Improved Puzzle-Solving During the PSAP/IntA Procedure

Regardless of puzzle difficulty (**a.**, 2 responses required; **b.**, 3-5 responses required; **c.**, 5-6 responses required), rats improved their performance during daily sessions (n=34; †, $p<0.0001$-0.05), making significantly more correct responses on trials 7-10 compared to trials 1-3 ($p<0.0001$-0.05) or 4-6 (puzzles 14-20; $p<0.05$). Graphs show mean ±SEM.

Figure 4: Drug-Seeking Behavior During PSAP/IntA

a. To determine changes in drug-seeking behavior with increasing PSAP/IntA experience (Session), while accounting for the increased number of puzzle responses required, behavior was analyzed as a rate (seeking responses per minute). Panel **a.** shows that the rate of drug-seeking increased across 4-weeks of cocaine self-administration (Puzzle-ON, black circles; †, $p<0.0001$-0.01, seeking days 14-20 vs 1-3 or 4-6). The rate of drug-seeking was significantly greater during "Puzzle-ON" periods, compared to "Puzzle-OFF" time outs (*, $p<0.0001$; white vs. black circles; $p<0.0001$-0.05, comparing each day). In a subset of rats (n=6), drug-seeking decreased when the tones that guided seeking behavior were omitted (No Tone, cross-hatched square; *, $p<0.05$ vs same rats during Puzzle-ON for sessions 14-20). **b.** Mistakes made while drug-seeking on each puzzle trial forced the rats to restart the puzzle from the beginning. Puzzles became harder to solve across sessions and, accordingly, the number of times the rats restarted each puzzle also increased (†, $p<0.0001$). n=34. Graphs show mean ±SEM.

Figure 5: Drug-Taking Behavior During PSAP/IntA

Panel **a.** shows the number of cocaine infusions during each min of the 5-min drug available period within daily sessions (Daily Trials 1-3, 4-6 and 7-10, horizontal axis) as a function of days of PSAP/IntA experience (open circles, the first 1-3 days of PSAP/IntA experience and closed circles after 14-20 days of PSAP/IntA experience). Although cocaine was available for a total of 5 min (FR1 schedule) after each puzzle completion on each trial, most of the infusions were

self-administered during the first min of drug-access (compare min 1, 2, 3, 4 and 5 during each of the trial blocks). During the first minute of cocaine access there was a significant increase in infusions administered both across sessions (Days 1-3 vs. 14-20; †, $p<0.0001$) and across trials for a given session (*, $p<0.05$). There was also a significant effect of trial number for sessions 1-3; animals took more cocaine in the first minute of availability on trials 4-6 and 7-10, relative to trials 1-3 ($p<0.001$-0.01). Rats also escalated cocaine intake for minutes 2-4 of drug-availability during sessions 14-20, relative to sessions 1-3 ($p<0.05$). Panel **b.** shows the average cocaine intake on the first daily trial across 4 PSAP/IntA blocks, and illustrates that rats escalated their cocaine intake across the four weeks of PSAP/IntA (†, $p<0.0001$). n=34. Graphs show mean ±SEM.

Figure 6: Individual Variation in Drug Self-Administration During PSAP/IntA

Rats were divided into two groups, either meeting 0-1 (n=10) or 2-3 (n=5) "addiction-criteria," as defined in the methods. **a-b.** During the acquisition of self-administration using the infusion criteria (IC) procedure, all rats increased responding for cocaine (**a.**, †, $p<0.0001$). However, 2-3 criteria rats were slower at completing either 20 or 40 drug infusions (**b.**, *, $p<0.01$, Effect of Group; †, $p<0.0001$, Effect of IC; $p<0.001$-0.01, 0-1 vs 2-3 criteria rats for either IC20 or IC40). **c.** Rate of drug-seeking during PSAP as a function of addiction criteria. The 0-1 and 2-3 criteria groups did not differ in the rate of drug-seeking prior to PSAP/IntA experience (Sessions 1-3). However, after PSAP/IntA experience (Sessions 14-20) rats meeting 2-3 addiction criteria showed a significant increase in drug-seeking, while rats meeting 0-1 criteria did not (†, $p<0.01$, days 1-3 vs 14-20 PSAP/IntA for 2-3 crit. rats; *, $p<0.05$, 0-1 vs. 2-3 crit. rats during PSAP/IntA days 14-20; Bonferroni), **d.** Rats meeting 2-3 addiction criteria escalated drug-intake (†, $p<0.01$, PSAP/IntA days 1-3 vs 14-20 for 2-3 crit. rats), whereas rats meeting 0-1 criteria did not significantly escalate cocaine intake. Graphs show mean ±SEM.

Figure 7: Individual Variation in Motivation for Drug

This figure summarizes changes in measures of cocaine demand and other addiction-like behaviors, as a function of PSAP/IntA experience (Baseline, BL vs. after PSAP/IntA experience, Post), and as a function of addiction criteria met (0-1 vs. 2-3 criteria). **a.** P_{max} is defined as the maximum amount rats were willing to pay (in effort) to maintain their preferred level of drug

consumption. P_{max} was increased in both 0-1 and 2-3 addiction criteria rats, but the magnitude of the increase was greater in the 2-3 criteria rats (†, p<0.001, BL vs. Post PSAP/IntA test for 2-3 crit. rats; *, p<0.001, 0-1 vs. 2-3 crit. rats during Post PSAP/IntA test). **b.** Elasticity of the demand curve (α) refers to how readily responding declines as cost (in effort) increases, and is normalized to the preferred level of consumption (Q_O) for each rat. Following PSAP/IntA experience all rats showed a decrease in α (that is, the demand curve became less elastic), indicating insensitivity to changes in drug price (†, p<0.01), and there were no group differences. **c.** There were no changes in the preferred level of cocaine consumption when cost was negligible (Q_O). **d.** Following PSAP/IntA, the 2-3 criteria rats were more willing to endure an electric shock to maintain their preferred level of cocaine consumption than 0-1 criteria rats, although these groups did not differ prior to PSAP/IntA experience (*, p<0.05, 0-1 vs. 2-3 crit. rats during Post PSAP/IntA test). **e.** Compared to rats meeting 0-1 addiction-criteria, rats meeting 2-3 criteria were more likely to continue responding on the taking lever during a single 90-min extinction session (*, p<0.05). **f.** During a test for cocaine-induced reinstatement, rats received one non-contingent infusion of cocaine (0/Ext, 0.2, 0.4, 0.8, 1.6 mg/kg) every 30 minutes. These infusions significantly increased responding on the taking lever (which had no consequence), regardless of addiction-criteria group (†, p<0.01). **g.** After the test for cocaine-induced reinstatement, rats underwent seven daily 2-hour extinction sessions. The 2-3 criteria rats responded more on the lever than the 0-1 criteria rats during extinction (*, p<0.0001) and there was also a significant effect of session (†, p<0.05; 2-3 criteria rats were different from 0-1 criteria rats on Ext-Ext2, but not Ext3-Ext7, Bonferroni). **h.** Next, on the test for cue-induced reinstatement (2-hours), lever presses resulted in cue-light presentation and concurrent activation of the infusion pump (not connected to rat) for 2 seconds. While all rats displayed cue-induced reinstatement, this effect was greatest in rats meeting 2-3 addiction-criteria (†, p<0.001-0.05, Ext7 vs CR for either 0-1 or 2-3 crit. rats; *, p<0.001, 2-3 vs 0-1 crit. rats for CR test). Rat criteria: 0-1 (n=10) or 2-3 (n=5). Graphs show mean ±SEM.

Figure 8: Dopamine and Drug-Seeking After PSAP/IntA Experience

The role of DA transmission in the DLS and NAc core was assessed after 4-weeks of drug self-administration using PSAP/IntA. Across three testing sessions, each rat was administered randomized bilateral microinjections (0.5 µl/side; DLS or NAc core) of saline (vehicle), 5µg, or 15µg of the DA receptor antagonist flupenthixol. Following infusion (1min) and diffusion (1min) of veh or drug, rats were returned to their home cage for 5 min, before being tested in their

respective operant chambers. On these sessions, drug-seeking was observed on a representative puzzle (#15). The total number of seeking responses was analyzed during the first puzzle-solving trial, before gaining access to the taking-lever. **a.** There was a significant interaction between the dose of flupenthixol and the brain injection site ($p<0.01$). Compared to vehicle, blockade of DA signaling in the NAc core reduced drug-seeking at both doses of flupenthixol (*, $p<0.05$). In contrast, 5 µg of flupenthixol injected into the DLS enhanced drug-seeking compared to either vehicle injections or 15 µg drug injections into the DLS (*, $p<0.05$), as well as compared to 5 µg of flupenthixol infused into the NAc core (*, $p<0.05$). Histological markings for microinjection sites into the NAc core (**b.**) or DLS (**c.**) are shown according to the Paxinos and Watson (2004) brain atlas. NAc core, n=8; DLS, n=7. Graphs show mean ±SEM.

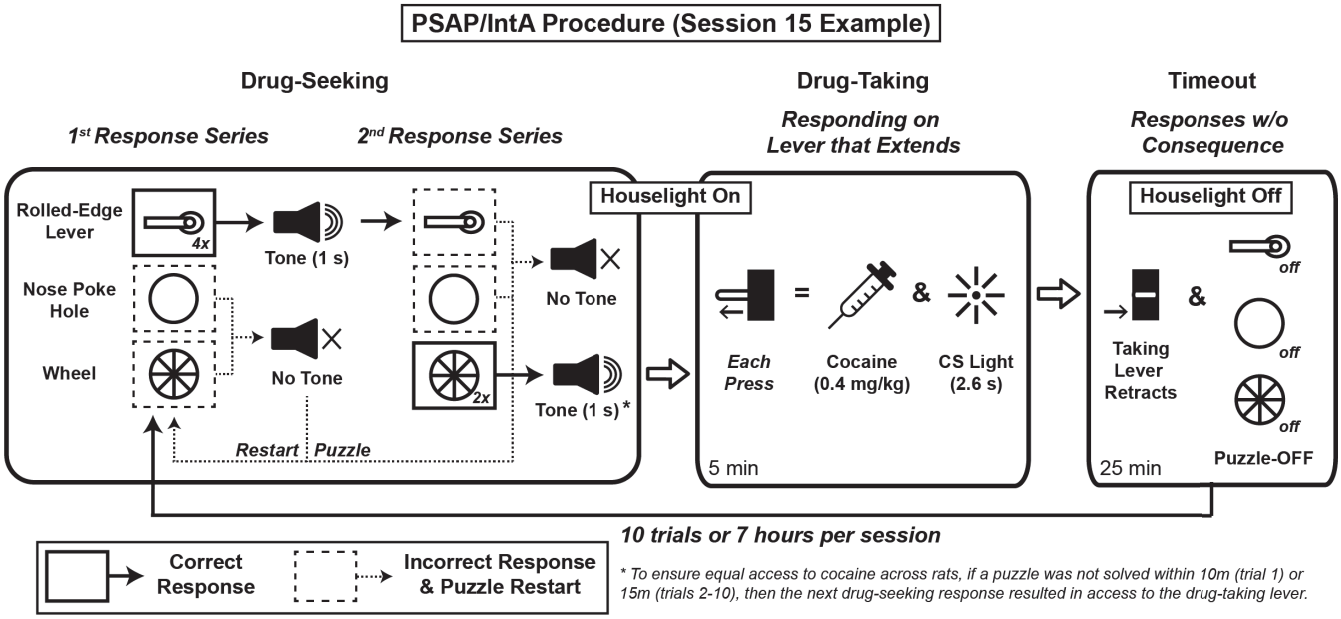
Session	1st Resp. Series	2nd Resp. Series
1	1 Nose Poke	X
2	1 Seeking Lever	X
3	1 Wheel Turn	X
4	1 Nose Poke	1 Seeking Lever
5	1 Wheel Turn	1 Nose Poke
6	1 Seeking Lever	1 Wheel Turn
7	3 Wheel Turns	2 Seeking Lever
8	2 Nose Pokes	3 Seeking Lever
9	4 Wheel Turns	1 Nose Poke
10	2 Seeking Lever	2 Wheel Turns
11	1 Nose Poke	2 Seeking Lever
12	4 Wheel Turns	1 Seeking Lever
13	2 Seeking Lever	2 Nose Pokes
14	3 Nose Pokes	2 Wheel Turns
15	4 Seeking Lever	2 Wheel Turns
16	3 Wheel Turns	3 Nose Pokes
17	3 Seeking Lever	2 Nose Pokes
18	4 Nose Pokes	2 Wheel Turns
19	3 Wheel Turns	3 Seeking Lever
20	4 Seeking Lever	1 Nose Poke
21	2 Nose Pokes	4 Wheel Turns
22	2 Wheel Turns	2 Seeking Lever
23	2 Seeking Lever	3 Nose Pokes
24	4 Wheel Turns	2 Nose Pokes
25	1 Seeking Lever	2 Wheel Turns
26	2 Nose Pokes	2 Seeking Lever
27	2 Wheel Turns	3 Nose Pokes
28	3 Nose Pokes	3 Wheel Turns

Stage 1: Food Self-Administration Training & Surgery			
Pellet Retrieval *(1-2 Days)*	**FR1 Taking** *(2 Days)*	**FR1-3 Seeking + FR1 Taking** *(9 Days Total)*	**Surgery + Recovery** *(Jugular Catheter & Intracranial Cannula)*

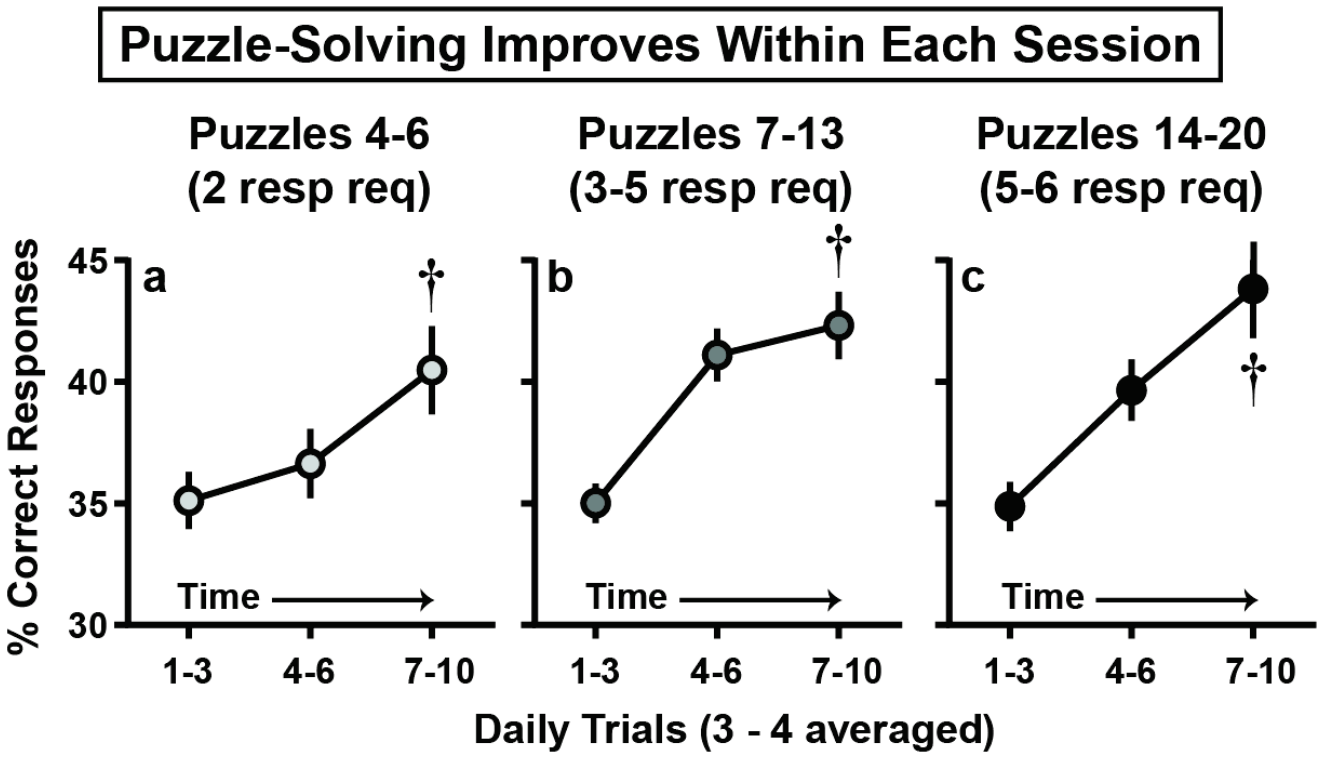
Stage 2: Cocaine Self-Administration Training & Tests for Addiction-Like Behavior				
Infusion Criteria 10 FR1 *(2 Days)*	**Infusion Criteria 20 FR1** *(3 Days)*	**Infusion Criteria 40 FR1** *(4 Days)*	**Behavioral Economic Threshold** *(5 Days)*	**Behavioral Economic Punishment** *(3 Days)*

Stage 3: Puzzle Self-Administration Procedure (PSAP) & Drug-Seeking Tests						
PSAP/IntA 1-20 *(5 Days/Week, 4 Weeks)*	**Seeking 1** *(Veh or Flu, 1 Day)*	**PSAP/IntA 21-22** *(2 Days)*	**Seeking 2** *(Veh or Flu, 1 Day)*	**PSAP/IntA 23-24** *(2 Days)*	**Seeking 3** *(Veh or Flu, 1 Day)*	**PSAP/IntA 25-26** *(2 Days)*

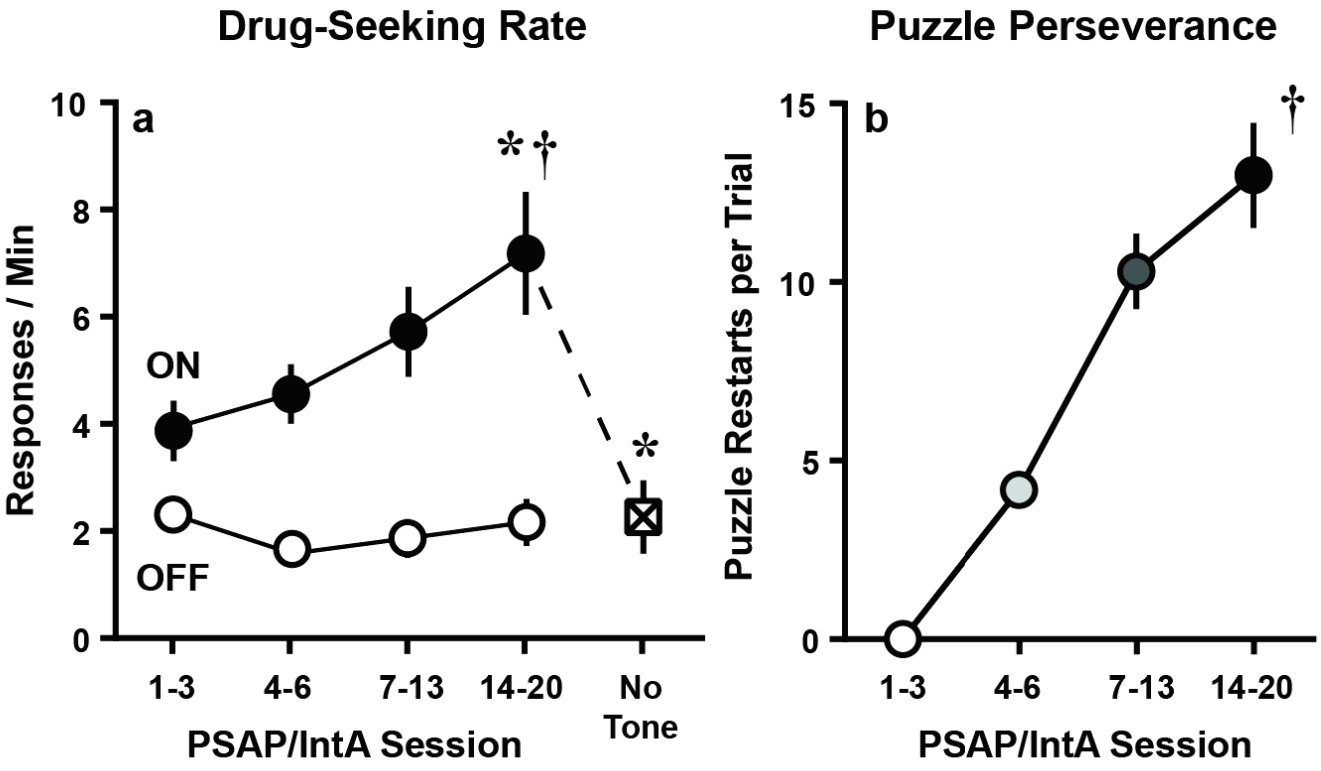
Stage 4: Final Tests for Addiction-Like Behavior					
Behavioral Economic Threshold *(2 Days)*	**Behavioral Economic Punishment** *(2 Days)*	**PSAP/IntA 27-28** *(2 Days)*	**SAL & COC Reinstatement** *(1 Day Each)*	**Extinction** *(7 Days)*	**Cue-Induced Reinstatement** *(1 Day)*

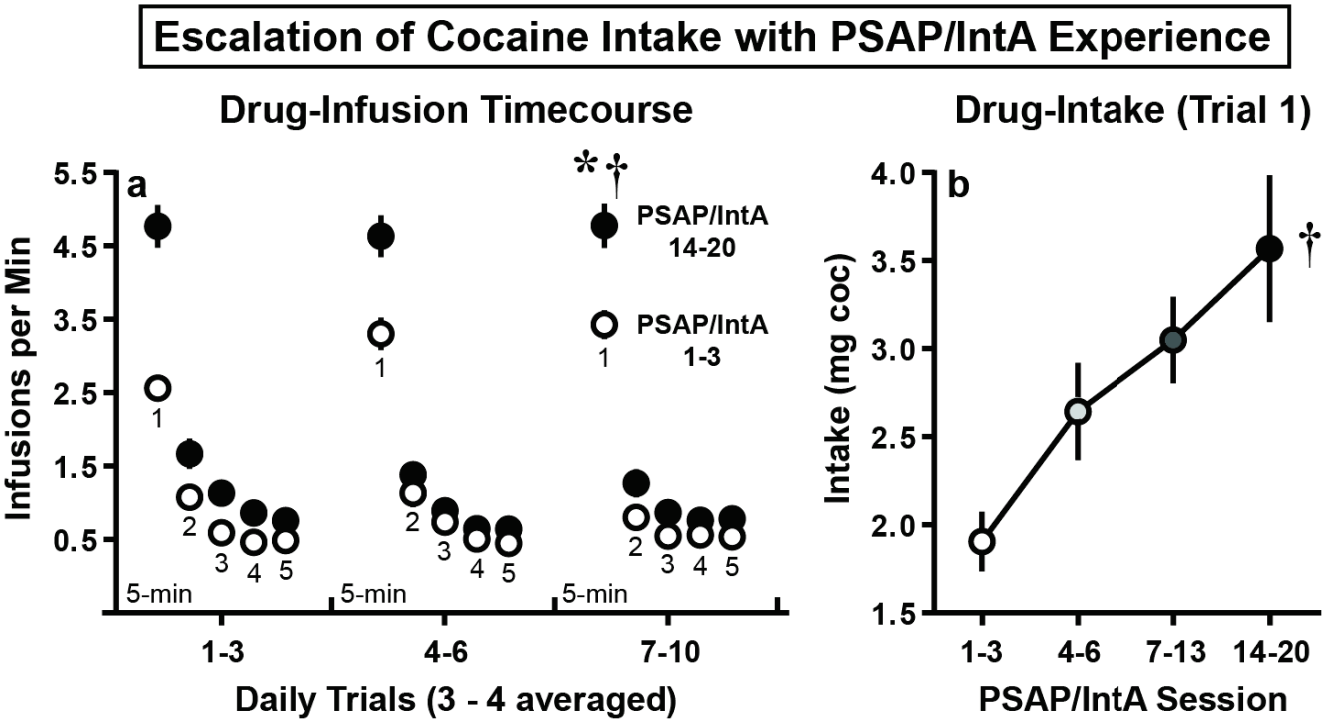


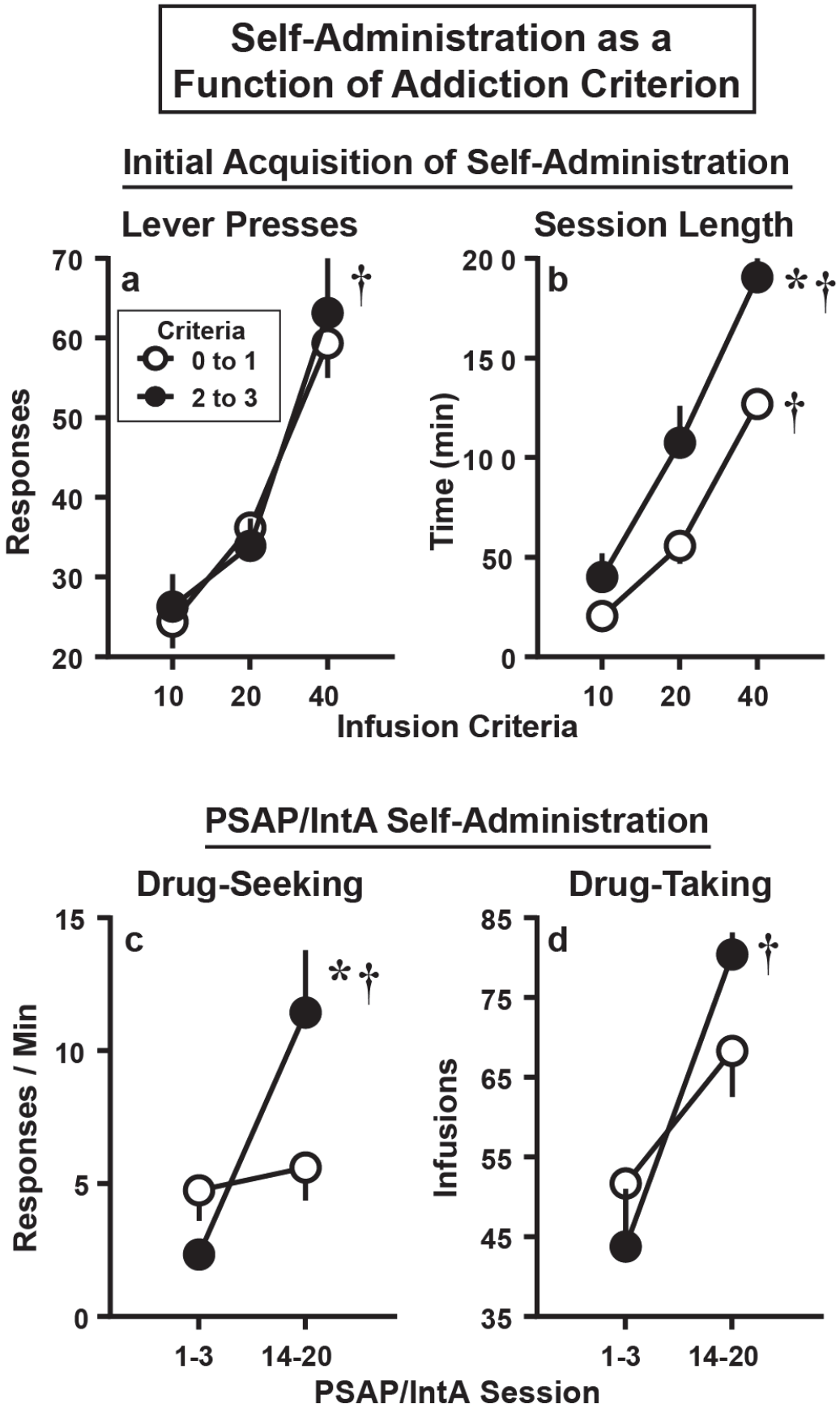
* To ensure equal access to cocaine across rats, if a puzzle was not solved within 10m (trial 1) or 15m (trials 2-10), then the next drug-seeking response resulted in access to the drug-taking lever.

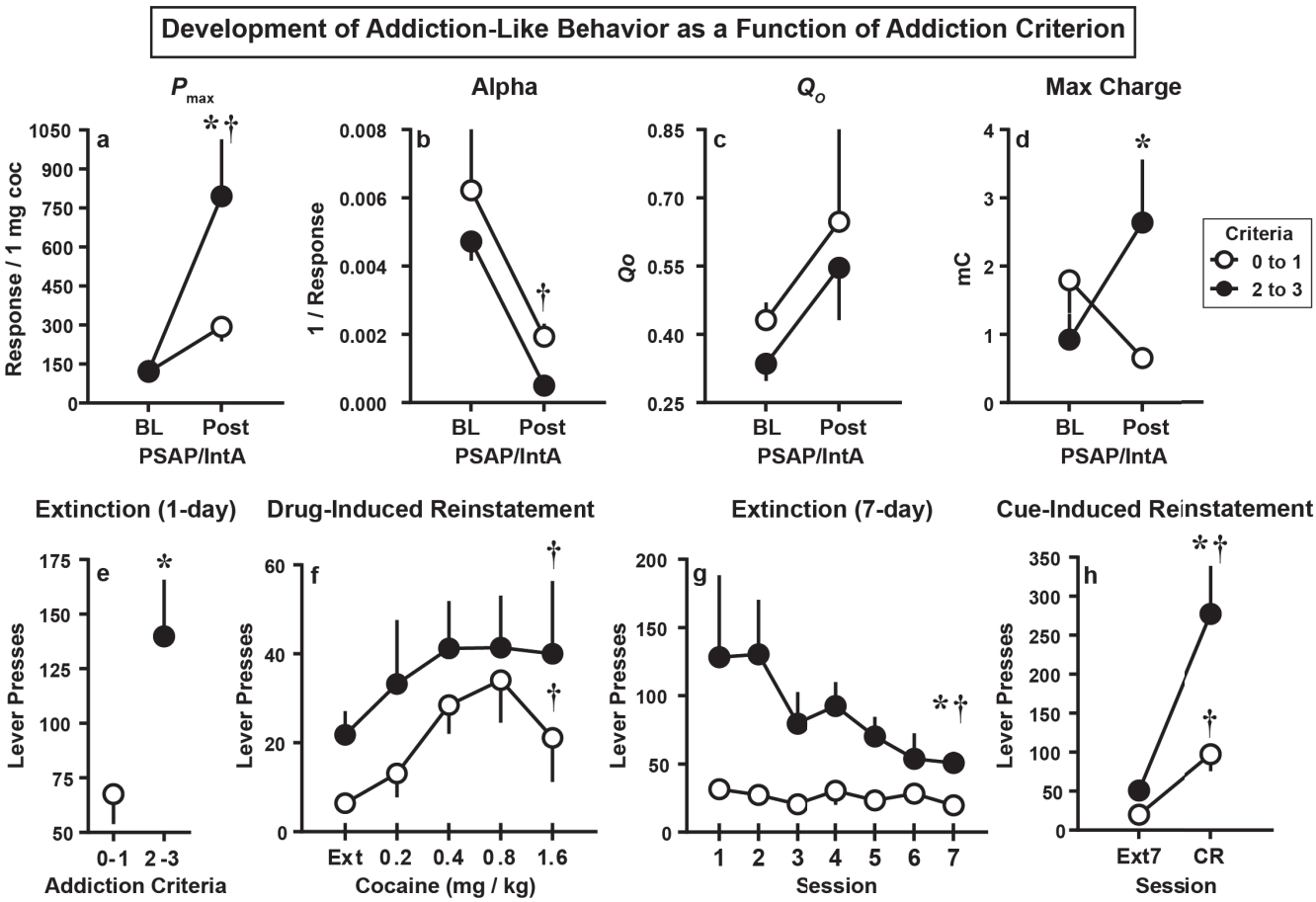


Drug-Seeking Increases with PSAP/IntA Experience, as Puzzles Increase in Difficulty









Flupenthixol in the NAc Core, but not in the DLS, Decreases Drug-Seeking