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Adolescent cocaine exposure enhances goal-tracking behavior and impairs hippocampal cell genesis selectively in adult bred low-responder rats

M. Julia García-Fuster^{1,*,#}, Aram Parsegian^{2,#}, Stanley J. Watson^{2,3}, Huda Akil^{2,3}, and Shelly B. Flagel^{2,3}

¹IUNICS/IdISPa, University of the Balearic Islands, Palma de Mallorca, Spain

²Molecular & Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, USA

³Department of Psychiatry, University of Michigan, Ann Arbor, USA

Abstract

Rationale—Environmental challenges during adolescence, such as drug exposure, can cause enduring behavioral and molecular changes that contribute to life-long maladaptive behaviors, including addiction. Selectively bred high-responder (bHR) and low-responder (bLR) rats represent a unique model for assessing the long-term impact of adolescent environmental manipulations, as they inherently differ on a number of addiction-related traits. bHR rats are considered “addiction-prone”, whereas bLR rats are “addiction-resilient”, at least under baseline conditions. Moreover, relative to bLRs, bHR rats are more likely to attribute incentive motivational value to reward cues, or to “sign-track”.

Objectives—We utilized bHR and bLR rats to determine whether adolescent cocaine exposure can alter their inborn behavioral and neurobiological profiles, with a specific focus on Pavlovian conditioned approach behavior (i.e. sign- vs. goal-tracking) and hippocampal neurogenesis.

Methods—bHR and bLR rats were administered cocaine (15 mg/kg) or saline for 7 days during adolescence (postnatal day, PND 33–39) and subsequently tested for Pavlovian conditioned approach behavior in adulthood (PND 62–75), wherein an illuminated lever (conditioned stimulus) was followed by the response-independent delivery of a food pellet (unconditioned stimulus). Behaviors directed towards the lever and the food cup were recorded as sign- and goal-tracking, respectively. Hippocampal cell genesis was evaluated on PND 77 by immunohistochemistry.

Results—Adolescent cocaine exposure impaired hippocampal cell genesis (proliferation and survival) and enhanced the inherent propensity to goal-track in adult bLR, but not bHR, rats.

*Corresponding author: M. Julia García-Fuster. IUNICS/IdISPa, University of the Balearic Islands, Cra. Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain. **Phone:** +34 971 259992. **Fax:** +34 971 259501. j.garcia@uib.es.

#These authors contributed equally to this publication.

Ethical standards

The experiments were carried out following the Guide for the Care and Use of Laboratory Animals: Eight Edition, revised 2011, published by the National Academy of Sciences, and all procedures were approved by the University of Michigan University Committee for the Use and Care of Animals.

Conflict of interest

The authors declare the absence of any competing financial interests concerning the content of this document.

Conclusions—Adolescent cocaine exposure elicits long-lasting changes in stimulus-reward learning and enduring deficits in hippocampal neurogenesis selectively in adult bLR rats.

Keywords

Adolescence; Cocaine; Pavlovian conditioning; Hippocampus; Neurogenesis

Introduction

Adolescence is a critical period of neurodevelopment across species (Spear 2000), and represents a unique period of susceptibility to the effects of drugs of abuse (Kelley et al. 2004; Spear 2011; Stanis and Andersen 2014). For example, an earlier age of onset for cocaine use has been associated with more pronounced structural alterations in reward-related regions of the brain (e.g., Wheeler et al. 2013) and enhanced drug-induced behavioral responses (e.g., Black et al. 2006; Zhu et al. 2016). Moreover, the enduring behavioral and molecular changes caused by adolescent drug use have been associated with the disruption of adaptive learning strategies in adulthood (e.g., Black et al. 2006; Sullivan et al. 2011) and the development of psychopathology (e.g., Volkow 2004; Marco et al. 2011; Zhu et al. 2016). Genetic predisposition is believed to play a role in either promoting or protecting individuals from these long-term maladaptive changes (e.g., Vink 2016; Morrow and Flagel, 2016), but the inherent difficulty in properly controlling for environmental factors, and the challenge of parsing cause from consequence makes this a particularly difficult relationship to elucidate in humans.

To more directly address how both genetic and neural factors might render an individual more or less susceptible to affective disorders and addiction, we manipulated genetically and temperamentally unique rat lines that are bred based on locomotor response to a novel environment (Stead et al. 2006; Flagel et al. 2010, 2014). Selectively bred high-responder (bHR) rats exhibit high levels of activity in response to novelty and bred low-responder (bLR) rats exhibit low levels of activity (Stead et al. 2006). Although these rats are bred based on locomotor response to novelty, we seem to have co-selected for several traits in the bred lines that are not necessarily apparent in outbred high- and low-responder rats (see Flagel et al. 2014; Belin et al. 2008, 2011; Belin and Deroche-Gamonet 2012). While bHR rats exhibit a number of addiction-related traits, including increased aggression and impulsivity, bLR rats are more anxious and more reactive to stress (see Flagel et al. 2014). Thus, these two phenotypes may represent two distinct paths to “addiction”, with inherent tendencies in bHRs (Flagel et al. 2016), and perhaps stress-induced drug-taking or drug-seeking behavior in bLRs (Flagel et al. 2014; Kabbaj et al. 2000).

One trait that consistently and robustly differs in bHR and bLR rats is the propensity to attribute incentive salience to reward cues (Flagel et al. 2010, 2014). That is, following a Pavlovian conditioning paradigm wherein lever presentation (conditioned stimulus, CS) is paired with a food reward (unconditioned stimulus, US), bHR rats develop a sign-tracking response consisting of approach toward and manipulation of the lever-CS upon its presentation, whereas bLR rats develop a goal-tracking response and approach the location of food delivery upon lever-CS presentation. Thus, bHR rats are sign-trackers and attribute

enhanced incentive motivational value or incentive salience to the lever-CS, and bLR rats are goal-trackers and treat the reward cue merely as a predictor. This relationship between the propensity to attribute incentive salience to reward cues and locomotor response to novelty is unique to these selectively-bred lines and not apparent in outbred rats (Flagel et al. 2009). Thus, this *a priori* knowledge of the traits that will be expressed in bHR and bLR rats provides an invaluable means to examine how environmental manipulations during adolescence, such as drug experience, might alter the expected phenotype in adulthood. Specifically, in the current study, we exploited bHR and bLR rats to assess whether adolescent cocaine exposure has an enduring impact on their inborn tendency to sign- and goal-track, respectively.

Previous studies in outbred rats have shown that exposure to ethanol during adolescence (McClory and Spear 2014) increases sign-tracking behavior, as does exposure to psychostimulants either during adolescence (Doremus-Fitzwater and Spear 2011) or in adulthood (Saddoris et al. 2016). While the specific neurobiological mechanisms underlying these drug-induced effects on sign-tracking behavior have not been elucidated, dopaminergic transmission in the NAc core is likely involved (Saddoris et al., 2016), as it has been shown to play a critical role in the sign-tracking, but not the goal-tracking response (Flagel et al. 2011; Saunders and Robinson 2012; Yager et al. 2014). Other brain regions have also been implicated in these behaviors (Haight and Flagel 2014; Haight et al. 2015), including the hippocampus (Fitzpatrick et al. 2016a, b). This is not particularly surprising since the hippocampus plays a fundamental role in regulating motivated behavior (Ito et al. 2005) and has been associated with differences in temperament (Oler et al. 2010) and environmental reactivity to stress (Lemaire et al. 1999; Clinton et al. 2011). In addition, it has been shown that systemic cocaine exposure impairs hippocampal neurogenesis in outbred rats (e.g., Yamaguchi et al. 2004; Mandym and Koob 2012). In adult bLR and bHR rats, we have shown that cocaine impairs different stages of neurogenesis, with a decrease in the birth of new progenitor cells in bLRs and a suppression of the survival of new neurons in bHRs. These phenotype-specific effects are believed to play a role in the bLR/bHR differences in cocaine-induced behavioral sensitization (García-Fuster et al. 2010). While the hippocampus has been implicated in addiction-related behaviors (Castilla-Ortega et al. 2016a), and in bHR/bLR differences in response to drugs of abuse (García-Fuster et al. 2010, 2011; Waselus et al. 2013; Flagel et al. 2014), the relationship between hippocampal cell fate and the propensity to attribute incentive salience to reward cues (i.e. sign- vs. goal-track) has never been assessed. Thus, we utilized bHR and bLR rats to examine the long-term effects of adolescent cocaine exposure on: 1) Pavlovian conditioned approach behavior, and 2) hippocampal cell genesis—using Ki-67 as a marker for cell proliferation, and BrdU for cell survival.

Materials and methods

Animals

Forty bHR and forty bLR adolescent (postnatal day, PND 25) male Sprague-Dawley rats from generation F38 were used in this study. Rats were housed in pairs in standard controlled environmental conditions (22 °C, 70% humidity, and 12-h light/dark cycle, lights

on at 06:00 h). Food and water were available *ad libitum*. All animal care and experimental procedures followed the *Guide for the Care and Use of Laboratory Animals: Eight Edition*, (revised in 2011, published by the National Academy of Sciences), and all procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

A timeline of the experimental design can be found in Fig. 1, with additional details below.

Locomotor response to novelty

Baseline locomotor response to novelty was assessed during adolescence (PND 25) by placing animals into clear acrylic 43 × 21.5 × 25.5 cm (high) cages equipped with infrared photocell emitters mounted 2.3 and 6.5 cm above a grid floor placed on top of standard bedding. All testing was performed between 9:30 AM and 12:30 PM in a different room from where the rats were housed. Lateral and rearing activity was monitored by a computer with a locomotion-testing rig and motion-recording software created in-house at the University of Michigan. The locomotor test session lasted for 60 min and data was collected in 5-min bins. For each rat, locomotion scores were calculated by adding the total number of lateral and rearing movements.

To assess whether cocaine exposure during adolescence (PND 33–39) affected locomotor response to a novel environment, the phenotype for which bHR and bLR rats were bred, rats were tested again during adulthood (PND 59) in an alternate novel environment. Rats were placed into the same clear acrylic cages as described above, but with no grid floor and Aspen bedding that was distinct in texture and odor from their homecage bedding.

Drug treatments

Rats were treated with 5-bromo-2'-deoxyuridine (BrdU; 50 mg/kg, i.p.; Calbiochem) for 3 days (PND 26–28) at 12-h intervals in order to label newly generated cells that differentiate and integrate in the hippocampus (Kempermann 2002; García-Fuster et al. 2010). Four days later, rats began a once daily regimen of either systemic cocaine (15 mg/kg) or saline that lasted for 7 consecutive days (PND 33–39). This drug regimen was previously shown to induce psychomotor sensitization in adult outbred (Gosnell 2005) and bHR rats (García-Fuster et al. 2010), and to impact brain markers in adulthood when given at this time-window during early-mid adolescence (see García-Cabrerizo et al. 2015; García-Cabrerizo and García-Fuster 2016).

Psychomotor activating effects of cocaine

Test chambers to assess cocaine-induced effects on locomotor activity were made of expanded PVC (33.02 × 68.58 × 60.96 cm tall) with stainless steel woven-wire cloth grid floors (30.48 × 60.96, 7.62 × 7.62 cm squares), placed above a metal catch tray. Behavior was recorded during habituation and following drug administration using USB 2.0 monochrome industrial video cameras (The Imaging Source LLC, Charlotte, NC) installed directly above each activity chamber. Digital videos recordings were made during testing using IC Capture 2.2 software (www.theimagingsource.com). After testing, these video files were transferred to an offline computer where subsequent analysis was performed using TopScan 2.0 software (Clever Sys, Inc.; Reston, VA, USA, www.cleversysinc.com). This

software is designed to utilize movement information from the animals' full body (e.g. nose, base of tail, etc.) in order to measure specific behaviors of interest including locomotor activity and stereotypy (see Flagel and Robinson 2007). Locomotor behavior was measured as the number of crossover bouts from one end of the chamber to the other based on user-defined areas of the activity chamber. Stereotypy was measured using the number of head-waving bouts (i.e. movement of the head from side-to-side while in place for at least 2 seconds).

On the first day of testing (D1, PND 33), rats were transferred to a new testing room where they were placed into custom-made activity chambers (Flagel et al. 2008). Here, rats were allowed to habituate for 2 h before receiving an injection of either cocaine (15 mg/kg, i.p.) or saline (0.9% NaCl, 1 ml/kg, i.p.). This was followed by 1 h of locomotor testing before being returned to their home cages. For the next 6 days (PND 34–38), rats were injected once daily with 15 mg/kg of cocaine or saline in an alternate room at the same time of day as the first test. Immediately following injections they were placed back into their homecages and returned to their colony room. On Day 7 (D7, PND 39), rats were again returned to the activity chambers where they were tested on Day 1 and allowed to habituate for 2 h before receiving an injection of either cocaine or saline followed by 1 h of behavioral scoring.

Pavlovian conditioning procedures

Sixteen standard test chambers (MED Associates, ST. Albans, VT, USA) were used for Pavlovian training as described recently in detail (Haight et al. 2015). Each chamber was located in a sound-attenuating box with a ventilating fan to mask outside noise. The test chambers were equipped with a white house light located at the top of the wall opposite to a food cup and a pellet dispenser, which were located in the middle of the front wall. An illuminated, retractable lever was located to either the right or left of the food cup at equal height and was counter-balanced across boxes to eliminate any side bias. The lever required a 10 g force to deflect and was recorded as a “lever contact.” The pellet dispenser delivered one 45 mg banana-flavored food pellet (BioServ®, Flemington, NJ, USA) into the food cup upon lever retraction. Head entry into the food receptacle was detected by an infrared photo beam located approximately 1.5 cm above the base of the food cup.

All training sessions were conducted between 1:00 PM and 5:00 PM. For 2 days prior to the start of training (PND 58–59) rats were handled by the experimenter and ~25 banana-flavored food pellets were placed into the rats' home cage to familiarize them with the food reward. During pre-training (2 sessions: PND 60–61) the house light was illuminated and the lever remained retracted for the duration of the session. Rats were placed individually into the test chambers where 50 food pellets were delivered one at a time into the food cup on a variable interval 30-s schedule. By the end of the second session rats were reliably retrieving the food pellets from the receptacle. Pavlovian training began the following day and continued for a total of 14 consecutive sessions with one session per day (PND 62–75). Each training session consisted of 25 trials, wherein an 8 s lever presentation (CS) was immediately followed by the response-independent delivery of a food pellet (US) into the food cup. The lever-CS was presented on a variable interval 90-s schedule (range 30–150 s), and the session lasted approximately 35–40 min. The following events were recorded by

MED Associates software: (1) the number of lever-CS contacts, (2) the latency to first lever-CS contact, (3) the number of head entries into the food receptacle (referred to as cup contacts) during presentation of the CS, and (4) the latency to first food cup entry upon lever presentation. The probability approach difference was calculated as the probability of lever vs. food cup contact [$\text{Prob}(\text{Lever}) - \text{Prob}(\text{Cup})$] in a given session (i.e. across 25 trials). These measures allowed us to quantify sign-tracking (lever-CS directed) and goal-tracking (food-cup directed) behavior.

Tissue collection

Rats were killed by rapid decapitation 2 days after the last Pavlovian conditioning session (PND 77). The extracted brains were cut sagittally and the left-half brain was quickly frozen in a -30°C isopentane solution and stored at -80°C until further processing. For each rat, $30\text{ }\mu\text{m}$ sections were cryostat cut and slide-mounted throughout the extent of the hippocampus and kept -80°C until processing for immunohistochemistry (IHC), as described below (see also García-Fuster et al. 2010).

Immunohistochemical analysis of cell genesis markers

Cell genesis markers (i.e., Ki-67 for recent cell proliferation and BrdU for cell survival) were analyzed by IHC in the hippocampus. Ki-67 labeling represents an index of recent cell proliferation as it labels cells within a cell cycle time of 25 h (i.e., cells born during PND 76–77) (Cameron and McKay 2001), at which point the rats were sacrificed. Therefore, Ki-67 allows us to measure how cocaine exposure during adolescence impacted the production of newly born cells in the hippocampus in adulthood. In contrast, systemic BrdU administration incorporates into new actively proliferating cells and labels cells at birth during the S-phase of the cell cycle (Wojtowicz and Kee 2006). Thus, we were also able to examine the number of BrdU-labeled cells that were born prior to any drug treatment (PND 26–28) and survived the extent of the 49–51 day experiment (PND 77) (Fig. 1).

Following a standardized protocol (García-Fuster et al. 2010), tissue was post-fixed in 4% paraformaldehyde and incubated for epitope retrieval. Sections were then rinsed with phosphate-buffered saline (PBS), washed in 0.3% peroxide, blocked with bovine serum albumin containing 1% goat serum and 0.05% Triton X-100, and incubated overnight with either polyclonal rabbit anti-Ki-67 (1:40,000; University of Michigan, USA) or polyclonal anti-BrdU (1:20,000; University of Michigan, USA). After PBS washes, sections were incubated in biotinylated anti-rabbit secondary antibody 1:1000 (Vector Laboratories, CA, USA) followed by Avidin/Biotin complex amplification (Vectastain Elite ABC kit; Vectors Laboratories) and diaminobenzidine for signal visualization. All sections were counterstained with cresyl violet before dehydration through graded alcohols, xylene immersion and coverslipping (Permount® mounting medium).

All slides were randomly coded by someone naïve to experimental conditions to ensure an unbiased quantification. Codes were broken after all slides were quantified and analyzed. The number of immunostained positive cells was counted in every 8th section throughout the septo-temporal extent of the hippocampus (-1.72 to -6.80 mm from Bregma) using a Leica DMR light microscope with a 63× oil objective lens in order to focus through the thickness

of the section (30 μm). For each rat the total number of proliferating (Ki-67+) or surviving (BrdU+) cells counted in the dentate gyrus was expressed in relation to the overall quantified area (mm^2) (Amrein et al. 2011; García-Cabrerizo et al. 2015), which was measured using a densitometer (GS-800 Imaging Calibrated densitometer, BioRad).

Statistical analysis

Locomotor activity data and cell genesis markers were analyzed using GraphPad Prism, Version 6. Results are expressed as mean values $\pm$ standard error of the mean (SEM). Two-way ANOVA followed by a multiple comparison test (Bonferroni) or Student's t-test, where appropriate, were used for the statistical evaluations. The level of significance was chosen as $p < 0.05$.

For Pavlovian conditioning procedures statistical analyses were performed using the SPSS Statistics program, version 21 (IBM, Armonk, NY, USA). Changes in PCA behavior across sessions, measured by contacts, latency to contact, probability of approach difference (food cup-lever), and probability of contact for either the lever-CS or the food cup, were evaluated using linear mixed-effects models (Verbeke and Molenberghs 2000), in which Treatment (cocaine vs. saline) and Session were treated as independent variables. The covariance structure was explored and modeled appropriately for each dependent variable. For all analyses significance was set at $p < 0.05$ and Bonferroni post-hoc analyses were used to correct for multiple comparisons when significant main effects or Treatment $\times$ Session interactions were found.

Results

Locomotor response to novelty

The locomotor activity (the sum of lateral and rearing movements) of bLR and bHR rats when placed in a novel environment at age PND 25 (baseline) and when placed in a different novel environment at age PND 59 is shown in Fig. 2. Repeated saline or cocaine exposure occurred on PND 33–39, after the initial assessment (PND 25) of locomotor response to novelty, but rats are grouped according to this treatment regimen for both novelty-induced locomotor tests. As expected, relative to bLRs, bHRs exhibited greater locomotor activity in response to a novel environment, regardless of treatment (Effect of Phenotype: $F_{1,36} = 44.35$, $p < 0.001$). When each phenotype was analyzed separately, a repeated measures two-way ANOVA with Age (PND 25 vs. PND 59) and Treatment (saline vs. cocaine) as factors showed that there was an overall effect of Age (bLRs: $F_{1,18} = 29.72$, $p < 0.001$; bHRs: $F_{1,17} = 65.45$, $p < 0.001$) on total locomotion, as both phenotypes showed increased activity in adulthood (PND 59) relative to adolescence ($p < 0.05$ vs. PND 25), regardless of treatment. This Age effect may be attributable to their larger size and ability to cover larger distances in the same amount of time in adulthood. Importantly, there was not a significant effect of Treatment (bLRs: $F_{1,18} = 0.27$, $p > 0.05$; bHRs: $F_{1,17} = 0.0003$, $p > 0.05$), nor was there a significant Age $\times$ Treatment interaction for either phenotype (bLRs: $F_{1,18} = 0.04$, $p > 0.05$; bHRs: $F_{1,17} = 0.05$, $p > 0.05$). Similar results were observed when analyzing lateral and rearing locomotion separately for each phenotype (data not shown). These results suggest

that a history of cocaine exposure during adolescence did not alter locomotor response to novelty in adult bLR and bHR rats.

Impact of adolescent cocaine exposure on behavioral sensitization in bLR and bHR rats

Given “baseline” differences in locomotor activity (effect of Phenotype for saline-treated groups across time points, $F_{1,9} = 39.20$, $p < 0.001$), bHRs and bLRs were analyzed separately for responses to cocaine. Acute (D1) and chronic (D7) cocaine administration increased psychomotor activity as measured by the number of crossover bouts in adolescent bLR rats (effect of Treatment: $F_{1,18} = 20.69$) relative to saline controls (Fig. 3a). However, there was no effect of Day ($F_{1,18} = 1.56$, $p > 0.05$) nor was there a Treatment $\times$ Day interaction ($F_{1,18} = 1.54$, $p > 0.05$) for bLRs. For bHRs, there was a significant effect of Treatment ($F_{1,18} = 17.85$, $p < 0.001$), Day ($F_{1,18} = 16.09$, $p < 0.001$) and a Treatment $\times$ Day interaction ($F_{1,18} = 7.48$, $p < 0.05$), revealing that cocaine, but not saline treatment, increased activity on D7 relative to D1 ($p < 0.01$, Fig. 3c). These data demonstrate that this cocaine treatment regimen elicited psychomotor sensitization in adolescent bHR, but not bLR, rats.

To determine whether bLR sensitization effects on locomotor activity were being masked by stereotypy, we assessed the number of bouts of stereotyped head waving in bLR (Fig. 3b) and bHR rats (Fig. 3d). There was a significant effect of Day ($F_{1,18} = 5.40$, $p < 0.05$), but no significant effect of Treatment ($F_{1,18} = 2.43$, $p > 0.05$), nor a Treatment $\times$ Day interaction ($F_{1,18} = 0.001$, $p > 0.05$) for bLRs (Fig. 3b). For bHRs, there was a significant effect of Treatment ($F_{1,18} = 6.60$, $p < 0.05$) and Day ($F_{1,18} = 8.59$, $p < 0.01$), but no Treatment $\times$ Day interaction ($F_{1,18} = 1.92$, $p > 0.05$). Thus, while acute cocaine elicited an increase in head-waving bouts in bHRs on D1 ($p < 0.05$), there was no evidence of a sensitized response using this measure of “stereotypy” for either bHR or bLR rats.

Impact of adolescent cocaine exposure on Pavlovian conditioned approach behavior in adult bLR and bHR rats

Sign- and goal-tracking performance across 14 consecutive training sessions for adult bLR and bHR rats that were treated during adolescence with repeated cocaine or saline are shown in Fig. 4. Given that bLRs and bHRs are known to differ in these behaviors, the effects of adolescent cocaine treatment on Pavlovian conditioned approach behavior were analyzed separately within each phenotype. Contrary to what we initially hypothesized based, in part, on previous findings (Doremus-Fitzwater and Spear 2011; McClory and Spear 2014; Saddoris et al. 2016), bLR rats exposed to cocaine during adolescence did not acquire a sign-tracking response as measured by the number of lever-CS contacts, probability to approach the lever-CS, and latency to contact the lever-CS (Fig. 4 a–c). However, adolescent cocaine exposure enhanced the inborn tendency of bLR rats to goal-track, as measured by the number of food cup contacts, probability to approach the food cup, and latency to approach the cup (Fig. 4 a–c). Linear mixed-effects models revealed a significant effect of Treatment ($F_{1,18} = 5.91$, $p < 0.05$), Session ($F_{13,18} = 62.02$, $p < 0.001$) and a Treatment $\times$ Session interaction ($F_{13,18} = 13.59$, $p < 0.001$) for food cup contacts (Fig. 4a). Pairwise comparisons revealed a significant difference between treatment groups during the later training sessions (Session 10, $p < 0.05$; Session 12, $p < 0.01$). For probability of food cup contact (Fig. 4b), a similar pattern was evident with a significant effect of Treatment ($F_{1,18} =$

4.55, $p < 0.05$) and Session ($F_{13,18} = 85.79$, $p < 0.001$) but no Treatment $\times$ Session interaction ($F_{13,18} = 1.33$, $p > 0.05$). Likewise, for latency to contact the food cup (Fig. 4c), there was a significant effect of Treatment ($F_{1,18} = 4.43$, $p = 0.05$) and Session ($F_{13,18} = 25.78$, $p < 0.001$) but no Treatment $\times$ Session interaction ($F_{13,18} = 1.72$, $p > 0.05$).

By comparison, exposure to cocaine (i.e., effect of Treatment) during adolescence did not alter the inborn tendency of bHR rats to sign-track as measured by the number of lever-CS contacts ($F_{1,18} = 0.21$, $p > 0.05$), probability to approach the lever-CS ($F_{1,18} = 0.17$, $p > 0.05$), and latency to contact the lever-CS ($F_{1,18} = 0.52$, $p > 0.05$) (Fig. 4d–f). Interestingly, there was also no effect of adolescent cocaine exposure on goal-tracking behavior in bHRs, as measured by the number of food cup contacts ($F_{1,18} = 3.08$, $p > 0.05$), probability to approach the food cup ($F_{1,18} = 3.55$, $p > 0.05$), and latency to approach the cup ($F_{1,18} = 3.08$, $p > 0.05$) (Fig. 4d–f).

In an additional analysis we compared the probability of approaching the lever-CS vs. the food cup during the CS period, averaged across training sessions (Sessions 1–14). Using this metric, positive numbers (scale from 0 to +1) indicate a tendency to sign-track, and negative numbers (scale from 0 to –1) indicate a tendency to goal-track (Flagel et al. 2010). The closer the value is to ± 1 , the stronger the tendency. The results show that bLR rats treated with cocaine during adolescence exhibit an increased tendency to goal-track relative to saline controls (Fig. 5a; Student's t-test, $p < 0.05$). In sum, all bLR rats developed a goal-tracking response, and this response was enhanced by cocaine treatment during adolescence. By contrast, all bHR rats developed a sign-tracking response regardless of adolescent cocaine treatment (Fig. 5b; Student's t-test, $p > 0.05$, Sal vs. Coc). Notably, the difference in response bias score is not closer to 1 for bHRs because it was averaged across all 14 conditioning sessions, not after they had reached asymptotic performance.

A more detailed analysis was conducted to examine the effects of cocaine exposure during adolescence on the topography of the conditioned response that emerged during Pavlovian training (Saunders and Robinson 2012). The percent of trials in which the bLR and bHR rats approached only the lever-CS, only the food cup or neither was evaluated across all Pavlovian conditioning sessions (Sessions 1–14). Cocaine administration during adolescence altered the response pattern of bLRs by increasing (+17%) the proportion of trials in which only the food cup was approached, and by decreasing (–17%) the proportion of trials in which no response was recorded across all training sessions (Fig. 5c; Student's t-test, $p < 0.05$, compared to saline controls). These results suggest that bLRs treated with cocaine in adolescence exhibit an increase in activity directed towards the food cup, relative to saline-treated bLRs, who spend more time doing nothing (i.e. not responding at the lever or the food cup). This food-cup directed activity was specific to cue presentation, as there were no group differences in responses directed towards the food cup during the intertrial interval (data not shown). Conversely, a history of adolescent cocaine exposure did not significantly alter the response pattern of bHR rats (Fig. 5d).

Impact of adolescent cocaine exposure on hippocampal cell genesis in adult bLR and bHR rats

The IHC experiments revealed that repeated cocaine exposure during adolescence (PND 33–39) impaired recent cell proliferation (23% decrease in Ki-67+Cells/Area; $p < 0.05$, Fig. 6a,c) and the survival of cells labeled by BrdU during adolescence (24% decrease in BrdU+Cells/Area; $p < 0.05$, Fig. 6a,c) in the hippocampus of adult bLR rats relative to saline-treated bLR controls. These results demonstrate long-term hippocampal cell loss as a result of adolescent cocaine exposure in bLR rats. By comparison, exposure to cocaine during adolescence did not alter hippocampal cell genesis (Ki-67+Cells/Area: Sal, 40 ± 3.2 vs. Coc, 38 ± 1.7 ; BrdU+Cells/Area: Sal, 117 ± 8.2 vs. Coc, 97 ± 9.8 ; $p > 0.05$ for both measures, Fig. 6b,d) in adult bHR rats.

Discussion

These data are the first to demonstrate that adolescent cocaine experience enhances goal-tracking behavior and reduces hippocampal cell genesis, and that it does so selectively in adult bLR rats. Others have previously shown that exposure to drugs of abuse (i.e., amphetamine, ethanol) in adolescence enhances the tendency to sign-track in outbred rats (Doremus-Fitzwater and Spear 2011; McClory and Spear 2014), and similar increases in sign-tracking behavior were reported in adult rats after cocaine self-administration (Saddoris et al. 2016). The seemingly discrepant results reported here are likely due to the use of selectively-bred bLR rats, which, unlike outbred rats, have an inherent predisposition towards goal-tracking behavior (Flagel et al. 2010), amongst other inborn traits (Flagel et al. 2014). For example, bLR rats also exhibit greater anxiety- and depressive-like behaviors following stressful experiences (i.e., Calvo et al. 2011; Stedenfeld et al. 2011). Relative to bHRs, bLRs exhibit a higher physiological stress response (e.g., stress-induced defecation; Clinton et al. 2014) and an attenuated corticosterone response to mild stressors, which appears to be a function of greater glucocorticoid receptor expression in the hippocampus (Clinton et al. 2008), a finding also reported in outbred HR/LR rats (Kabbaj et al. 2000). These bHR-bLR differences in HPA activity likely influence the dopamine system (e.g., Rougé-Pont et al. 1998), which, in turn, could alter the response to cocaine (i.e., absence of bLR cocaine sensitization, García-Fuster et al. 2010) as well as stimulus-reward learning (Flagel et al. 2010, 2011). Thus, it is possible that the physiological stress effects of repeated cocaine treatment during adolescence, combined with the inherent sensitivity of the HPA-axis and increased negative affect in bLR rats, alters brain organization and development in such a way that enhances goal-tracking behavior. That is, the impact of adolescent cocaine and the prolonged withdrawal experience in bLRs may override the neurobiological changes that have been shown to elicit enhanced incentive salience attribution, as measured by sign-tracking, perhaps due to a more direct consequence on adult hippocampal function. In support, excessive stress or repeated cocaine exposure has been shown to result in impaired adult hippocampal neurogenesis (Gould and Tanapat 1999; García-Fuster et al. 2010), and adult rats with a history of cocaine exposure during adolescence show increased anxiety-like behavior and an increased number of damaged neurons in the hippocampus (Zhu et al. 2016). Additional experiments will be needed to evaluate how other types of stress exposure, besides adolescent cocaine, impact goal-tracking behavior in bLR rats.

The unique inborn neurobiological attributes of bLRs could explain why, as opposed to bHR rats, we did not see psychomotor sensitization in the current study or in prior studies with similar experimental conditions (i.e., García-Fuster et al. 2010). While adolescent cocaine exposure induced psychomotor sensitization in bHR rats, it did not further increase sign-tracking behavior in these rats, nor did it alter goal-tracking behavior. The lack of increase in sign-tracking behavior beyond their inherent predisposition might suggest a ceiling effect for this behavioral response in bHRs. Furthermore, once the sign-tracking response is acquired, it is difficult to disrupt (Flagel et al. 2009; Haight et al. 2015), which might also explain why there was not a change in goal-tracking behavior in bHRs.

An increased propensity to sign-track, or attribute incentive salience to reward cues, has been associated with a number of addiction-related behaviors (e.g., Tomie 1996; Flagel et al. 2009; Robinson et al. 2014). Likewise, adolescent drug exposure has been associated with an earlier onset and increased propensity for addiction (Spear 2011; Stanis and Andersen 2014). Thus, we expected adolescent cocaine exposure to enhance sign-tracking behavior, but the current findings are incongruent with this notion. These unexpected results may indeed be due to inherent predispositions in the selectively-bred rat lines, as discussed above; yet, it should also be noted that recent data suggests that sign- and goal-trackers may be equally susceptible to addiction (Kawa et al. 2016), but via different (genetic and neural) pathways. Thus, while sign-trackers are more responsive to discrete drug-associated cues (Flagel et al. 2011; Yager et al. 2014) and more likely to exhibit cue- and drug-induced reinstatement of drug-seeking behavior relative to goal-trackers (Saunders and Robinson 2010, 2011) after relatively brief periods of cocaine self-administration, both phenotypes exhibit addiction-related behaviors following prolonged intermittent access to cocaine (Kawa et al. 2016). In addition, goal-trackers have been shown to exhibit enhanced reinstatement of drug-seeking behavior in response to drug-associated contexts (Saunders et al. 2014). Similar to outbred goal- and sign-trackers, we have postulated that bLRs and bHRs may represent two distinct paths to “addiction”. That is, bLRs may be more susceptible to stress-induced drug-taking or drug-seeking behavior, while bHRs are more likely to exhibit addiction-related behaviors under “baseline” conditions (Flagel et al. 2014, 2016). Indeed, bLRs are not completely resilient to the effects of adolescent cocaine, as the same treatment regimen used in the current study has been shown to elicit behavioral sensitization in response to a single cocaine injection in adulthood (Parsegian et al. 2016). Furthermore, there is evidence to suggest that the two potential paths to “addiction” in bLRs and bHRs are likely mediated via distinct neural pathways (Wasselus et al. 2013).

The hippocampus is one structure that has been implicated in the differences in “emotionality” between bHRs and bLRs (e.g., Clinton et al. 2008). While the hippocampus is known to be particularly sensitive to stress (e.g., Gould and Tanapat 1999), and involved in several aspects of addiction liability (Castilla-Ortega et al. 2016a), including drug-context memory (e.g., Meyers et al. 2006) and relapse to drug-seeking (e.g., Vorel et al. 2001), the current findings are the first to investigate the relationship between hippocampal cell genesis and Pavlovian conditioned approach responses, specifically as a consequence of adolescent cocaine exposure. Adult hippocampal cell genesis (i.e., cell proliferation and survival; Cameron and McKay 2001) represents one form of hippocampal plasticity that contributes to the brain’s ability to process and respond to challenges (Mandyam and Koob 2012), such

as drug exposure (e.g., Noonan et al 2008, 2010) during adolescence. More specifically, cocaine and other drugs of abuse given in adulthood diminish cell genesis (García-Fuster et al. 2011; reviewed in Mandyam and Koob 2012). This phenomenon, as measured by decreased cell proliferation, was shown in adult bLR rats exposed to the same cocaine treatment regimen as that used in the current study, an effect still observed following 14 days of abstinence (García-Fuster et al. 2010). Similar to the current findings, bLR rats given this treatment regimen in adulthood also failed to show signs of psychomotor sensitization (García-Fuster et al. 2010). Contrary to the present results, however, no change was detected when evaluating cell survival following cocaine treatment in adulthood (García-Fuster et al. 2010). This discrepancy may be related to the different age of the animals (adolescence vs. adulthood) at which cocaine was administered, or to the different length of the abstinence period. To the best of our knowledge, all previous studies evaluated cell genesis after giving a drug treatment regimen in adulthood and followed by up to four weeks of forced withdrawal (e.g., Noonan et al. 2008; see review in Mandyam and Koob 2012). In contrast, in the present experiment, we administered cocaine during adolescence (PND 33–39) and evaluated cell genesis (both cell proliferation and survival) in adulthood (PND 77) following an extensive withdrawal period (38 days) following the last cocaine injection. Therefore, the present results are the first to demonstrate that adolescent cocaine exposure can cause, selectively in bLR rats, life-long deficits on the number of adult-born progenitor cells and the survival of BrdU-labeled mature cells 49–51 days after they were labeled in adolescence. Interestingly, although hippocampal-dependent learning is unlikely to have impacted the number of BrdU+ cells, as they were more than 30 days old at the beginning of the Pavlovian conditioning procedures, it could have regulated its proliferative response. Thus, the observed decrease in hippocampal cell proliferation in cocaine-treated rats might also be attributed to an altered neuroplastic response to learning.

These data are consistent with the view that decreased cell genesis (i.e., proliferation and survival, Mandyam and Koob 2012), which has been shown to enhance vulnerability to addiction and relapse (Noonan et al. 2010), compromises the restructuring of the hippocampus during cocaine withdrawal, leading to a decrease in the formation of new connections. Such dysregulation of hippocampal cell genesis could influence how a rat responds to a conditioned stimulus. Further, there is evidence to suggest that hippocampal cell genesis participates in the contextual regulation of addiction-related behaviors. For example, ablating neurogenesis in the hippocampus during forced abstinence following cocaine self-administration increases the propensity for context-dependent reinstatement (Noonan et al. 2010), while reducing the number of adult-born hippocampal neurons increases time spent in a cocaine-paired context in a test of conditioned place preference (Castilla-Ortega et al. 2016b). These data are congruent with the fact that goal-trackers appear to be more susceptible to reinstatement in response to drug-associated contexts (Saunders et al. 2014) and that bLR rats are more sensitive to changes in hippocampal neurogenesis. Taken together, the relationship between adolescent cocaine exposure, the regulation of hippocampal cell genesis, and the enhanced expression of goal-tracking behavior suggested by the current findings, warrants further investigation.

In summary, these results suggest that the hippocampus participates in the regulation of goal-tracking behavior, and that adolescent cocaine exposure can produce enduring

behavioral and neurobiological changes, at least in some individuals. Indeed, these data highlight individual variation in the consequences of adolescent cocaine experience, producing severe detriments, such as compromised brain development and reduced hippocampal function in adulthood in vulnerable individuals, rendering them more susceptible to psychopathologies, including addiction.

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Fig. 1.

Experimental design. PND: post-natal day; ST: sign-track; GT: goal-track.

Fig. 2.

Locomotor response to novelty in (a) bLR and (b) bHR rats. Data represents mean $\pm$ SEM of locomotion (total activity, which is the sum of lateral and rearing movements) on post-natal day (PND) 25 (Baseline, $n=10$ per group) and PND 59 following adolescent (PND 33–39) repeated treatment with cocaine (15 mg/kg, 7 d, $n=10$) or saline ($n=10$) for each phenotype. A repeated measures two-way ANOVA with Age (PND 25 vs. PND 59) and Treatment (saline vs. cocaine) as factors showed that there was an overall effect of Age on total locomotion for bLR and bHR rats. At least $*p < 0.01$ for PND 59 vs. PND 25.

Fig. 3.

Adolescent cocaine exposure produces behavioral sensitization in bHR but not bLR rats.

Mean $\pm$ SEM mm of (a, c) crossover bouts and (c, d) head-waving bouts for bLR (a–b) and bHR (c–d) rats following acute (D1: PND 33) and repeated (D7: PND 39) saline (Sal, n=10) or cocaine treatment (Coc, 15 mg/kg, n=10). Although cocaine treatment increased psychomotor activity as measured by crossover bouts relative to saline controls (on D1 and D7) for both phenotypes, only bHR rats showed evidence of sensitization ($\psi p < 0.01$; Effect of day: D1 vs. D7). *** $p < 0.001$ and * $p < 0.05$ as compared to Sal-treated control group.

Fig. 4.

Adolescent cocaine exposure does not alter sign-tracking behavior in either bHR or bLR rats, but enhances goal-tracking behavior in adult bLR rats only. Food cup-directed behavior (goal-tracking) and lever-CS directed behavior (sign-tracking) across 14 days (PND 62–75) of Pavlovian conditioning approach training for bLR (a–c) and bHR (d–f) rats treated with cocaine (n=10) or saline (n=10) during adolescence (PND 33–39). Data are expressed as the mean $\pm$ SEM of (a, d) the number of contacts, (b, e) the probability of approach and (c, f) the latency of first approach to the lever-CS or the food cup. ** $p < 0.01$ and * $p < 0.05$ as

compared to Sal-treated control group (Linear mixed-effects models followed by Bonferroni post hoc pairwise comparisons).

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Fig. 5.

(a) Adolescent cocaine exposure reduces the difference in the probability to approach the lever-CS vs. the food cup in bLR rats treated with cocaine ($n=10$) or saline ($n=10$) during adolescence (PND 33–39). (b) Adolescent cocaine exposure did not alter the response pattern of bHR rats. Data are expressed as the mean $\pm$ SEM probability of approach to the lever-CS minus the probability of approach to the food cup (scale from 0 to -1 or 0 to $+1$). * $p < 0.05$ as compared to Sal-treated control group (Student's t -test). (c) Effect of adolescent treatment (Sal vs. Coc) on response topography (proportion of Cup trials, Lever Trials or None trials) across sessions 1–14 in adult bLR rats (Student's t -test, $p < 0.05$, Sal vs. Coc for Cup trials and None trials). (d) Adolescent cocaine exposure did not change the response topography across sessions 1–14 in adult bHR rats.

Fig. 6.

Adolescent cocaine exposure reduces hippocampal cell genesis in adult bLR rats.

Quantitative analysis of Ki-67+ and BrdU+ cells by area (mm²) in the left dentate gyrus of the hippocampus of bLR (a) and bHR (b) rats expressed as the mean $\pm$ SEM of the total number of quantified cells by area. Groups of treatment: Sal (n=10/phenotype) and Coc (n=10/phenotype). * $p < 0.05$ as compared to bLR-Sal-treated control group (Student's *t*-test). Representative IHC of Ki-67+ (left panels, brown labeling in blue granular cell layer) and BrdU+ (right panels, dark brown labeling in light brown granular layer) cell labeling following treatment (Sal vs. Coc) during adolescence (PND 33–39) in bLR (c) and bHR (d) rats. For each treatment group, an image was taken with a light microscope using a 63 $\times$ objective lens to identify individual cells. Scale bar: 30 μ m.