Behavioral/Systems/Cognitive

Altered Attention and Prefrontal Cortex Gene Expression in Rats after Binge-Like Exposure to Cocaine during Adolescence

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Illicit use of drugs frequently begins and escalates during adolescence, with long-term adverse consequences. Because it is increasingly accepted that neural development continues through adolescence, addiction research has become more invested in understanding the behavioral and molecular consequences of early exposure to drugs of abuse. In a novel binge administration paradigm designed to model the pattern of human adolescent drug use, we administered ascending doses of cocaine or saline during a 12-d developmental period [postnatal day 35 (P35) to P46] corresponding to human adolescence. During adulthood (P70), rats treated with this regimen displayed increased responsiveness to the stimulant effects of cocaine. Adult rats also displayed abnormally rapid shifts in attention when performing an attentional set-shifting task, which measures the ability to shift attention between stimuli and whose performance requires an intact prefrontal cortex (PFC). Treatment with cocaine during adolescence also caused acute alterations in the expression of genes encoding cell adhesion molecules and transcription factors within the PFC. Furthermore, we observed decreases in histone methylation, which may indicate a role for chromatin remodeling in the observed changes in gene expression patterns. These findings suggest that exposure to cocaine during adolescence has far-reaching molecular and behavioral consequences in the rat PFC that develop over time and endure long after drug administration has ceased.

Key words: **medial prefrontal cortex; attentional set-shifting task; cell adhesion; adolescence; cocaine; gene expression**

Introduction

Substance use in adolescence is a reliable predictor of substance abuse in adulthood (Merline et al., 2004). During adolescence, the developing brain may be vulnerable to long-lasting modifications by drug exposure (Andersen, 2003). Studies in rodents show that early developmental exposure to psychostimulants causes behavioral alterations in sensitivity to drugs of abuse and natural rewards that endure into adulthood (Andersen et al., 2002; Bolanos et al., 2003), raising the possibility that early exposure to psychostimulants can have long-term negative consequences (Carlezon and Konradi, 2004). However, in some studies, the adolescent brain appears resilient, showing few long-term effects of substance abuse (Santucci et al., 2004). The contradictory nature of these studies underscores the need for more detailed investigations of these behaviors and their molecular foundations.

Studies on cocaine exposure in adult humans and rodents have linked the medial prefrontal cortex (PFC), an area critical for cognition and executive function, to the development and maintenance of addiction-related behaviors (Piazza et al., 1991; McGregor et al., 1996; Weissenborn et al., 1997), compulsive drug-taking (Goldstein and Volkow, 2002), behavioral sensitization (Pierce and Kalivas, 1997), and craving and relapse after withdrawal (Childress et al., 1999; McFarland and Kalivas, 2001). Dysfunction of the frontal cortex can result in behaviors such as impulsivity, impaired decision making, and disinhibition (Arnsten and Goldman-Rakic, 1998; Bechara et al., 2001), which are often observed in chronic cocaine users (Bolla et al., 1999, 2003). Although such studies are invaluable for our understanding of the PFC and addiction-related behaviors, they do not specifically address the consequences of drug exposure on the developing adolescent brain, which is in the final stages of synapse formation and pruning (Casey et al., 2000; Spear, 2000). We designed studies to examine the long-term neurobiological and behavioral effects of cocaine exposure during adolescence on the medial PFC.

Models of binge cocaine administration that mimic human adolescent patterns of intake are currently not available. Typically, adolescents begin taking drugs at lower doses and increase their frequency of intake over time (O'Malley et al., 1985). We designed a rodent model of adolescent "binge" use that approximates this unique pattern, based on reports examining cocaine use patterns in teenagers (O'Malley et al., 1985), and on an adult binge administration model for rodents (Unterwald et al., 1994),

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Table 1. Example of a shift from the odor dimension to the medium dimension

	Dimension		Exemplar combinations	
Phase (day)	Relevant	Irrelevant	Positive	Negative
sD (1)	Odor		**Vanilla**	Jasmine
cD (1)	Odor	Medium	**Vanilla**/Sm Foil Balls	Jasmine/Lg Foil Balls
			Vanilla/Lg Foil Balls	Jasmine/Sm Foil Balls
cD-Rev (1)	Odor	Medium	**Jasmine**/Lg Foil Balls	Vanilla/Sm Foil Balls
			Jasmine/Sm Foil Balls	Vanilla/Lg Foil Balls
IDS (2)	Odor	Medium	**WhiteMusk**/Whole Tubing	TeaRose/Chopped Tubing
			WhiteMusk/Chopped Tubing	TeaRose/Whole Tubing
IDS-Rev (2)	Odor	Medium	**TeaRose**/Whole Tubing	WhiteMusk/Chopped Tubing
			TeaRose/Chopped Tubing	WhiteMusk/Whole Tubing
EDS (2)	Medium	Odor	**Sm Glass Beads**/Mandarin	Lg Glass Beads/Patchouli
			Sm Glass Beads/Patchouli	Lg Glass Beads/Mandarin
EDS-Rev (2)	Medium	Odor	**Lg Glass Beads**/Mandarin	Sm Glass Beads/Patchouli
			Lg Glass Beads/Patchouli	Sm Glass Beads/Mandarin

The reinforced exemplar is shown in bold. Pairs of exemplars used for each dimension are also shown. The same exemplar pair is presented no more than two consecutive times. Sm, Small; Lg, large.

with slight modifications after consultations with a clinical psychologist specializing in adolescent drug use. We administered ascending doses of cocaine in a binge pattern over a 12 d developmental period corresponding to human adolescence, from postnatal day 35 (P35) to P46. We verified the expression of cocaine-induced locomotor sensitization in adulthood and examined behavior in the Attentional Set Shift Task (ASST). Performance in this task is impaired by damage to the PFC (Birrell and Brown, 2000; McAlonan and Brown, 2003). In parallel, we analyzed gene expression patterns and examined the effect of cocaine on histone methylation, demonstrating the molecular impact cocaine exposure has on the developing PFC, and providing a possible mechanism for shifts in gene transcript levels.

Materials and Methods

Subjects

One hundred sixteen male Sprague Dawley rats (Taconic Farms, Germantown, NY) weighing 75–100 g on arrival were group housed in sets of five to six per 48.3 × 26.7 × 20.3 cm clear plastic cage. The colony room was humidity controlled and maintained on a 12 h light/dark cycle (lights on at 7:00 A.M.). Behavioral testing took place during the light phase of the cycle. For all rats, food and water were available *ad libitum* from the day of arrival on P29 through the end of the cocaine or saline injection series at P46. From 3 d before behavioral testing until the end of testing, rats that performed the ASST were maintained on a restricted diet of 16–25 g of food per day, with water available *ad libitum*. Rats in all other studies had *ad libitum* access to food and water throughout. All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and McLean Hospital's Institutional Animal Care and Use Committee guidelines.

Drugs

Cocaine HCl (Sigma, St. Louis, MO) was dissolved in 0.9% sterile saline solution to create final concentrations of 5, 7.5, 10, and 15 mg/kg. Sterile saline solution (0.9%) served as the vehicle solution in all experiments.

Adolescent binge cocaine administration

Cocaine was administered to rats in an ascending dose binge pattern over a period of 12 d starting on P35, corresponding to a period of early adolescence in humans (Andersen, 2003). Rats received three injections per day of either 0.9% saline (vehicle) or 5 mg/kg cocaine at P35–P36, 10 mg/kg at P37–P39, a 2 d abstinence period, and 15 mg/kg cocaine at P42–P46. All injections were given intraperitoneally, 1 h apart, with the first injection occurring at 10:00 A.M.

Experiment 1: Effects of cocaine exposure during adolescence on the expression of cocaine-induced locomotor sensitization in adulthood

Locomotor sensitization is defined as the enhanced responsiveness to the locomotor-activating effects of a drug. Sensitization is often observed after repeated intermittent drug administration and is thought to involve the PFC (Wolf et al., 2004). To determine whether rats exposed to the binge cocaine protocol during adolescence expressed behavioral sensitization in adulthood at 10 d (P56) or 24 d (P70) posttreatment, we examined their locomotor response to a challenge injection of cocaine versus one of saline (vehicle). To avoid the motivational aspects of cocaine withdrawal that last several days beyond termination of binge cocaine, we chose 10 d as our earliest testing time point, because it has been shown that no motivational signs of cocaine withdrawal are present 9 d after binge cocaine (Goussakov et al., 2006).

Apparatus. Locomotor activity was quantified in automated Plexiglas activity chambers, 43.2 × 43.2 × 30.5 cm (length × width × height) contained within sound-attenuating boxes (Med Associates, St. Albans, VT). Photocell beam breaks were used to track distance traveled and vertical rearing counts. Ventilation fans were on throughout the test sessions.

Testing. Thirty-four rats treated with either cocaine or saline during the adolescent period were tested for the expression of cocaine-induced locomotor sensitization either 10 d (P56) or 24 d (P70) after their last injection of cocaine or saline. These ages correspond to the late adolescent/early adult and adult periods, respectively, in humans (Andersen, 2003). On day 1 of testing, rats were habituated to the locomotor chamber for a period of 1 h before receiving a challenge injection of 0.9% saline. On day 2, rats were habituated to the chamber for 1 h before a challenge injection of 7.5 mg/kg cocaine. After each challenge injection, rats were immediately placed back into the locomotor chamber for 1 h.

Data analysis. Distance traveled and vertical rearing behaviors were analyzed for each time point using a two-factor repeated-measures ANOVA (treatment group by challenge day). Paired *t* tests were used for all *post hoc* analyses. Data from outliers, defined as subjects whose performance fell above or below 2 SDs from the mean, were removed from the analysis. Thirty-three rats were included in the final analyses.

Experiment 2: Effects of cocaine exposure during adolescence on ASST performance in adulthood

In a modified version of the ASST (Birrell and Brown, 2000), adult rats (P56 and P70) performed a series of discriminations using stimuli from two (instead of three) distinct perceptual dimensions, odor and digging medium (Table 1). An attentional set, or bias, is formed for the perceptual dimension with a history of reinforcement. In rats with an established attentional bias, the medial PFC is required to effectively shift attention to a different perceptual dimension [extradimensional shift (EDS) (e.g., odor to medium)] (Birrell and Brown, 2000). In contrast, shifts in attentional sets to new stimuli within the same perceptual dimension [intradimensional shift (IDS) (e.g., from one odor to a second odor)] or "reversals" of already-learned discriminations do not require the medial PFC (Birrell and Brown, 2000; McAlonan and Brown, 2003). We examined the number of trials it took for a subject to make the correct "shift" in an attentional set, after a change in the stimuli or reward contingencies presented. The criterion for each phase of the task was six consecutive correct trials.

Apparatus. The testing apparatus was a semitranslucent plastic bin (63.5 × 41.9 × 23.2 cm) that was divided into two equal-sized compartments (31.5 × 41.9 cm), separated by a black, nontransparent divider. At the start of a trial, a rat was placed in one compartment and the divider was raised, allowing access to two terracotta pots (internal diameter, 10 cm; depth, 8.5 cm) located adjacent to each other. The location of the reinforced pot within the compartment was randomized for each trial. After the rat entered into the compartment with the pots, the divider was put in place. Each pot was scented with a perfume oil (The Body Shop, Wake Forest, NC) and contained distinct digging media in which the animal could dig for a food reward (one-half Frosted Cheerio, stale; General Mills, Minneapolis, MN). Pairs of media were composed of the

same material and only differed in shape or size to ensure that the smell of the media was not used for discrimination. The bottoms of all pots were filled with purified paraffin ~12 mm high, and covered by a layer of Cheerios held in place by a mesh screen that made the Cheerios inaccessible to the rat. This was done to ensure that any potential "Cheerio odor" was present in both reinforced and nonreinforced pots.

Habituation/training. On the days before testing, rats were trained to make a "dig" response and find the Cheerio buried in the medium. A single unscented pot filled with polypropylene pellets was continuously rebaited until the rat approached and dug in <10 s after raising the divider. Then, animals were trained on two simple discriminations (sDs) designed to familiarize the rats with the different perceptual dimensions of the stimuli they would later discriminate. The exemplars used for these trials (odor of lavender vs ananya; medium of Eppendorf tube bottoms vs Eppendorf tube lids) were not used in later tests. The first four trials of a session were defined as exploratory trials, in that the rat was permitted to dig in both pots, regardless of whether the first choice was the correct (positive) or incorrect (negative) pot. During subsequent trials, once a "dig" was executed in one pot, the other pot was removed to prevent digging in both pots within one trial. A "dig" was defined as any distinct displacement of the digging medium with either the paw or the nose. Based on this definition, a rat could investigate the scents on the pot or the medium in the pot by sniffing or even touching the medium, before executing an actual dig response.

Testing. Forty-four rats treated with cocaine or saline during the adolescent period performed the ASST either 10 d (P56) or 24 d (P70) after their last injection of cocaine or saline. To avoid the satiety and fatigue that resulted from long test sessions with comparatively young animals during pilot studies, rats performed the task over 2 d, instead of 1 d as was originally published by Birrell and Brown (2000). Testing, therefore, lasted for ~2 h per rat per day. On day 1, rats performed a sD in which pots with either two distinct odors or two distinct digging media were presented for discrimination. Only one contingency within a perceptual dimension was reinforced (positive). After reaching criterion, rats were presented with a compound discrimination (cD), where the second perceptual dimension was introduced in the presence of the same positive and negative stimuli as the sD. The aim of this component of the task was to maintain focus on the stimuli reinforced during the sD, and ignore the presence of stimuli from the new perceptual dimension. After completion of the cD, rats performed a reversal (cD-Rev) in that the previously negative stimulus became the positive stimulus. This did not entail a shift in perceptual dimension, but rather an alteration in the stimulus–reward value of the stimuli presented (Table 1). Beginning on day 2, an IDS was initiated. During this phase of the task, entirely novel stimuli were presented, but the relevant perceptual dimension from the previous day remained the same. The attentional set formed in the phases before the IDS should bias a subject's learning toward the previously reinforced perceptual dimension. After the IDS, rats performed another stimulus reversal (IDS-Rev), followed by an EDS. In this case, a novel set of stimuli was presented, but the previously irrelevant perceptual dimension became the relevant dimension. The final discrimination was another stimulus–reward reversal (EDS-Rev), similar to the cD-Rev and IDS-Rev mentioned above (Table 1).

Because the possible exemplar combinations were too numerous to permit complete counterbalancing, exemplars were always used in pairs (Table 1). No two rats within the same treatment group received the same combinations through the phases of testing; however, rats from different treatment groups were matched for the pairs of stimuli presented.

Data analysis. The number of trials to criterion was measured for each phase of the task. A priori comparisons were performed on the dimensional shift (IDS vs EDS) phases of the task using a two-factor, repeated-measures ANOVA (treatment by IDS/EDS performance). A three-factor, repeated-measures ANOVA was used to determine any significant main effects or interactions. Treatment (cocaine/saline), discrimination phase (sD/cD/IDS/etc.), and shift type (odor to medium/medium to odor) were the factors included in this analysis.

Experiment 3: Effects of cocaine exposure during adolescence on gene expression in the medial PFC

Gene expression after cocaine administration is dynamic with different groups of genes showing distinct temporal expression profiles. In the past, we found that the immediate-early gene c-*fos* peaks between 30 and 45 min after an acute cocaine or amphetamine injection, whereas the gene for the neuropeptide prodynorphin (encoding the endogenous opioid dynorphin) peaks between 12 and 24 h (Konradi et al., 1994; Cole et al., 1995; Konradi et al., 1996). Because we were interested in the accumulation of genes coding for neuropeptides and structural proteins and not immediate-early genes, we compared patterns of mRNA transcript levels in cocaine- and saline-treated rats 22 h (P47) after the final injection. Twenty-two hours after the final injection also corresponds to the time when the first injection would have occurred on that day (or 24 h after the first injection). We also investigated gene expression changes 24 d (P70) after treatment, a time when we expected sensitization to occur in rats after long-term abstinence from cocaine. The 22 h time point provided insight into the gene expression pattern during chronic cocaine administration, whereas the 24 d time point provided insights into the long-term molecular consequences of early cocaine exposure.

Gene array analysis. Twenty-two rats were used in this experiment, 11 of which were treated with cocaine (6 killed 22 h after the last injection; 5 killed 24 d after the last injection) and matched with an equal number of saline-treated rats. Samples from individual rats were hybridized to individual arrays for a total of 22 arrays. Brains were extracted, quickly frozen in 2-methylbutane cooled to −30°C, and stored at −80°C. The area of the brain corresponding to the medial PFC [cingulate cortex 1, prelimbic cortex, and infralimbic cortex; from bregma, anterioposterior (AP), +4.2 to +2.2] (Paxinos and Watson, 1986) was dissected. RNA was extracted from ~10–15 mg of tissue using the RNAgent kit (Promega, Madison WI). RNA quality was assessed in an analytical gel, and 6 μg of total RNA was used for cDNA synthesis with the SuperScript double-stranded cDNA synthesis kit (Invitrogen, Carlsbad, CA). *In vitro* transcription (IVT) was performed with an IVT kit (Affymetrix, Santa Clara, CA). Biotinylated RNA was hybridized to the RAE230A array, and washing and staining were performed according to company protocol (Affymetrix). The Affymetrix RAE230A array contains over 15,000 probe sets representing 4700 full-length genes and 10,500 expressed sequence tags (ESTs); each probe set contains 11 perfectly matched 25-mer oligonucleotides, and the same number of one-mismatch oligonucleotides to provide values for nonspecific binding.

Quality control criteria. Tissue preparation and RNA extractions were performed in a single batch by the same investigator. All quality control criteria defined by Affymetrix were met by the samples, and no significant differences between the experimental groups were observed in these criteria. The average percentage "present" call across all arrays was 58.6 ± 2.7%, and the 3′/5′ GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and β-actin ratios were 1.3 ± 0.4 (mean ± SD), and 2.1 ± 0.4, respectively. Noise (2.6 ± 0.3) and scaling factor (1.6 ± 0.3; target value, 150) were not different between treatment groups.

Data analysis. A number of different programs were used for data analysis: RMAExpress (Bolstad et al., 2003; Irizarry et al., 2003) was used for quantile normalization and background correction to compute expression levels for all probe sets. DNA-Chip Analyzer (dChip, version 1.3; http://www.biostat.harvard.edu/complab/dchip/) (Li and Wong, 2001) and Gene Microarray Pathway Profiler (http://www.genmapp.org/) (Dahlquist et al., 2002) were used to group regulated genes into functional annotations. GeneChip Operating Software (GCOS) (Affymetrix) was used for scanning and to obtain quality control data. Expression values were $\log_2$-transformed for all analyses.

Real-time reverse-transcriptase PCR (quantitative PCR). Complementary DNA was synthesized from 1 μg of total RNA, with the SuperScript First-Strand Synthesis System for real-time quantitative PCR (Invitrogen), and an oligonucleotide deoxythymidine primer. A primer set for each gene was designed with the Primer3 software (www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi.), for amplicons of 100–200 base pairs. Melt curve analysis and polyacrylamide gel electrophoresis were used to confirm the specificity of each primer pair. The iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) was used for the experiment which

was performed with a MyiQ real-time PCR detection system (Bio-Rad) in a volume of 20 μl, with 4 μl of 1:10 diluted cDNA samples and 0.3 μM primers. The PCR cycling conditions were initially 95°C for 5 min followed by 39 cycles of 94°C for 10 s, 57°C for 15 s, and 72°C for 20 s. Data were collected between 72 and 84°C depending on amplicon melt temperature. A melt curve analysis was performed at the end of each quantitative PCR (Q-PCR) experiment, from 60 to 95°C. Dilution curves were generated for each primer pair in every experiment by diluting complementary DNA from a vehicle sample to a final concentration of 1.00, 0.1, and 0.01. The logarithm of the dilution values was plotted against the cycle values for the standard curve. Blanks were run with each dilution curve to control for cross-contamination. Dilution curves, blanks, and samples were run in duplicate. Reported values were normalized to the internal standards β-actin (GenBank accession number NM_031144) and general transcription factor 2β (GenBank accession number NM_031041), neither one of which was regulated in the gene-array or Q-PCR analysis.

Experiment 4: Effects of cocaine exposure during adolescence on histone methylation in the medial PFC

Posttranslational modifications of histone proteins such as methylation, acetylation, ubiquitination, and phosphorylation can increase or decrease the condensation of the chromatin structure, thereby allowing or limiting access to the DNA and altering levels of gene transcription (Wu and Grunstein, 2000; Berger, 2002; van Leeuwen and Gottschling, 2002). Previous research has shown that cocaine administration can induce alterations in chromatin structure in the striatum (Kumar et al., 2005). To examine epigenetic modifications by cocaine in the medial PFC, we performed Western blot analysis of two methylation sites of histone H3, lysine 4 and lysine 27.

Immunoblots. Frozen tissue from adolescence binge cocaine- and saline-treated rats ($n = 14$) killed 22 h after their last injection was punched from the medial prefrontal cortex (from bregma, AP: +2.2 to + 4.2) (Paxinos and Watson, 1986), weighed, and added to Laemmli buffer to get a final concentration of 1 mg of tissue/25 μl of buffer. Samples were briefly sonicated and centrifuged for 5 min. The supernatant was removed, heated to 80°C for 5 min, and loaded onto precast NuPAGE 10% Bis-Tris buffered polyacrylamide gels (Invitrogen) for separation by gel electrophoresis. Proteins were transferred to a polyvinylidene fluoride transfer membrane (PerkinElmer Life Sciences, Boston, MA) and blocked in blocking buffer (5% nonfat dried milk in TBS with 0.1% Tween 20) for 1 h. Blots were washed and incubated in primary polyclonal antibody (1:10,000 anti-Lys^4-trimethlyated-H3, 1:10,000 anti-Lys^{27}-trimethlyated-H3 and 1:20,000 anti-actin) at 4°C overnight. After four 15 min washes, blots were exposed to secondary antibody (1:5000 peroxidase-labeled anti-rabbit IgG, polyclonal) for 2 h and washed for 1 h. Blots were developed using Western Lightning Chemiluminescence Plus Reagents (PerkinElmer Life Sciences) and analyzed using a Kodak Image Station 440CF. The trimethylated-H3 proteins were analyzed in individual blots. Bands were observed at 17 kDa for the trimethylated proteins and at 40 kDa for actin. Unpaired *t* tests were used to analyze differences between cocaine- and saline-treated groups.

Results

Experiment 1: Effects of treatment with cocaine during adolescence on the expression of cocaine-induced locomotor sensitization in adulthood

Distance traveled and vertical rearing behavior in an open field were quantified during 1 h sessions, after rats received intraperitoneal injections of saline on day 1 and 7.5 mg/kg cocaine on day 2. Distance traveled and vertical rearing behaviors were also quantified during 1 h habituation sessions before saline and cocaine challenge sessions. Behavior on habituation day 1 provided a measure of locomotor response to novelty, because the rats had not been exposed to the locomotor chambers before sensitization testing. Thirty-three of 34 rats were included in the analyses. Of those included in the analyses, 7 cocaine-treated and 9 saline-treated subjects were tested 10 d (P56) after their final injection, and 9 cocaine-treated and 8 saline-treated subjects were tested 24 d (P70) after their final injection of cocaine or saline.

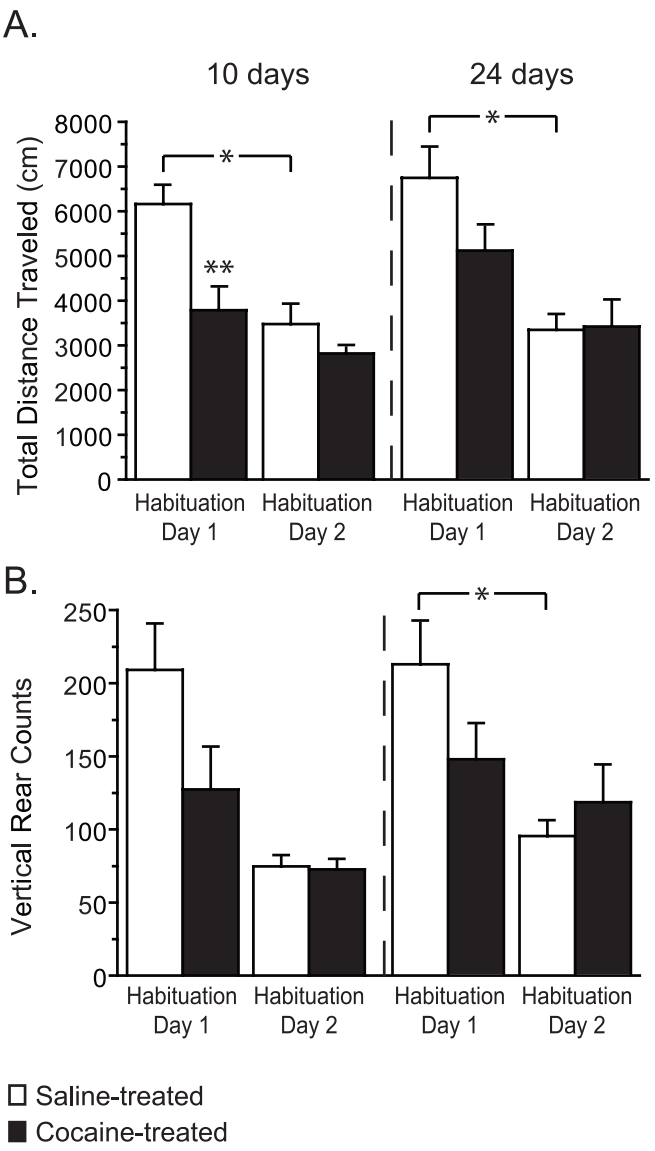

Figure 1. Performance during 1 h habituation sessions in rats treated during adolescence with cocaine and saline and tested 10 d (cocaine, $n = 7$; saline, $n = 9$) and 24 d (cocaine, $n = 9$; saline, $n = 8$) after treatment. ***A***, Total distance traveled. ***B***, Vertical rear counts. Values represent the mean + SEM. *$p \leq 0.05$; **$p \leq 0.005$.

Habituation days 1 and 2

Rats treated with saline during adolescence traveled significantly greater distances on habituation day 1 than habituation day 2 when tested 10 and 24 d posttreatment (10 d, $F_{(1,14)} = 10.67$, $p = 0.006$; 24 d, $F_{(1,15)} = 7.55$, $p = 0.02$) (Fig. 1*A*). A similar pattern was observed in vertical rearing behavior at 24 d posttreatment ($F_{(1,15)} = 6.48$; $p = 0.02$) and approached significance at 10 d posttreatment ($F_{(1,14)} = 4.08$; $p = 0.06$) (Fig. 1*B*). Thus, control rats showed increased exploratory behavior in a novel environment. In contrast, rats treated with cocaine during adolescence failed to show this heightened locomotor response to a novel environment. Indeed, compared with saline-treated controls, rats treated with cocaine during adolescence were significantly less active on habituation day 1 when tested 10 d after cocaine exposure (distance traveled, $t_{14} = -3.94$; $p = 0.002$), and ap-

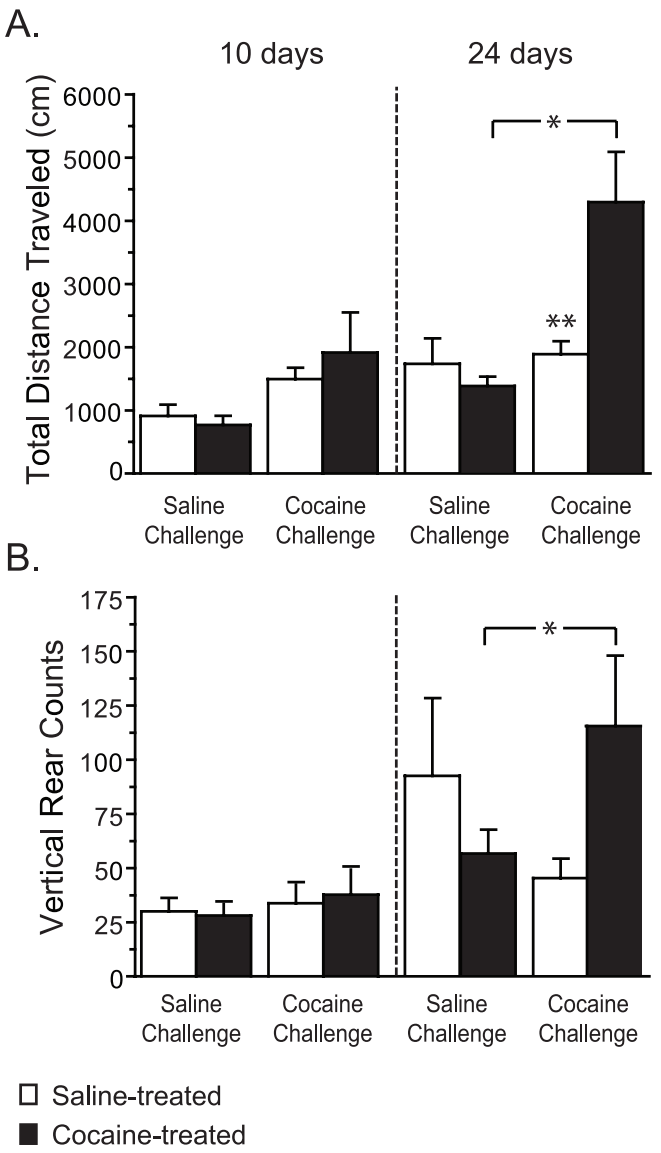

Figure 2. Performance during 1 h cocaine and saline challenge sessions in rats treated during adolescence with cocaine and saline and tested 10 d (cocaine, $n = 7$; saline, $n = 9$) and 24 d (cocaine, $n = 9$; saline, $n = 8$) after treatment. ***A***, Total distance traveled. ***B***, Vertical rear counts. Values represent the mean + SEM. $^*p \leq 0.05$; $^{**}p \leq 0.005$.

proached significance 24 d (distance traveled, $t_{15} = -1.94$; $p = 0.07$) after cocaine exposure (Fig. 1*A*).

Saline and cocaine challenges

Ten days after their final injection, rats treated with cocaine during adolescence did not show a sensitized locomotor response to a 7.5 mg/kg injection of cocaine. However, all rats traveled a greater distance in response to a cocaine challenge injection compared with saline injection (main effect of treatment; $F_{(1,14)} = 7.20$; $p = 0.02$), indicating that cocaine did have locomotor-activating effects (Fig. 2*A*,*B*, left). In contrast, 24 d after cocaine treatment, rats were significantly more active in response to a 7.5 mg/kg challenge dose of cocaine (distance traveled, $F_{(1,15)} = 11.18$; $p = 0.004$; vertical rears, $F_{(1,15)} = 4.24$; $p = 0.05$), as shown by a significantly greater distance traveled ($t_{15} = 2.912$; $p = 0.01$) and a strong trend toward more vertical rears ($t_{15} = 2.03$; $p = 0.06$) than saline-treated rats (Fig. 2*A*,*B*, right).

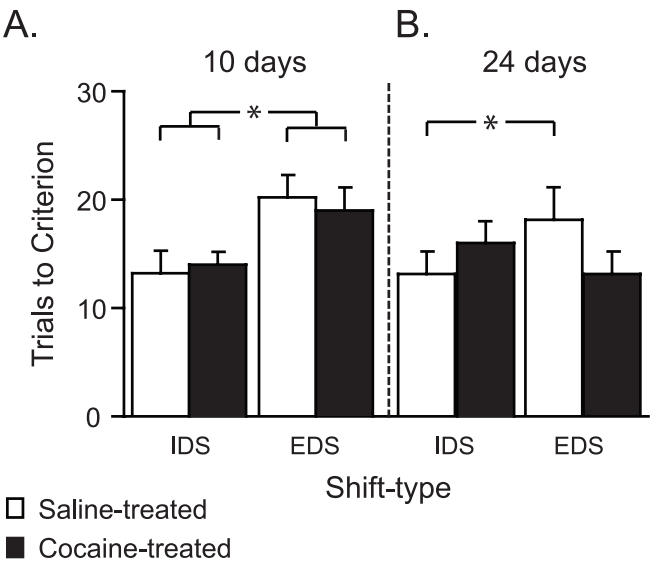

Figure 3. A priori comparisons of the trials to reach criterion during IDS versus EDS phases of the ASST in rats treated during adolescence with cocaine and saline. ***A***, Performance 10 d after treatment (cocaine, $n = 12$; saline, $n = 11$). ***B***, Performance 24 d after treatment (cocaine, $n = 11$; saline, $n = 10$). Values represent the mean + SEM. The criterion was as follows: six consecutive trials correct. $^*p \leq 0.05$.

Experiment 2: Effects of treatment with cocaine during adolescence on ASST performance in adulthood

Of the 46 rats that performed the ASST, 44 were included in the final analyses. Two rats were excluded because of failure to complete all phases of the task. Of those included in the analyses, 12 cocaine-treated and 11 saline-treated subjects were tested 10 d (P56) after their final injection, and 11 cocaine-treated and 10 saline-treated subjects were tested 24 d (P70) after their final injection of cocaine or saline.

When tested 10 d posttreatment, all adult rats took significantly more trials to shift their attention from one perceptual dimension to another (EDS), compared with shifting attention within the same perceptual dimension (IDS) (a priori main effect of IDS/EDS, $F_{(1,21)} = 7.56$; $p = 0.01$) (Fig. 3*A*). Because only the EDS phase required a shift of attention to a previously irrelevant perceptual dimension, the ASST paradigm was validated by the significant difference in trial numbers between the EDS and IDS phases. At 24 d posttreatment, the same pattern was observed in adult rats treated with saline during adolescence; however, adult rats treated with cocaine during adolescence shifted their attention more rapidly between stimuli of different perceptual dimensions, showing no difference between the numbers of trials taken to complete the IDS versus EDS phases of the task (a priori main effect of IDS/EDS, $F_{(1,19)} = 0.54$; $p =$ NS; interaction, $F_{(1,19)} = 5.33$; $p = 0.03$). *Post hoc* analysis (paired *t* test) confirmed that, whereas controls took significantly more trials to complete the EDS versus the IDS ($t_9 = -2.45$; $p = 0.03$), rats exposed to cocaine during adolescence showed no significant difference in IDS and EDS performance ($t_{10} = 1.01$; $p = 0.34$) (Fig. 3*B*).

Although the overall ANOVA on all phases of the task did not identify significant interactions, several main effects were observed (Fig. 4*A*,*B*). In general, all rats took more trials to complete the reversal phases of the task compared with the simple, compound, and IDS discriminations (10 d, $F_{(6,19)} = 10.42$; $p < 0.0001$; 24 d, $F_{(6,16)} = 4.88$; $p = 0.002$). We observed no main effects of shift type (medium to odor vs odor to medium) for rats tested at either time point, confirming that one dimensional shift type was not more difficult than the other.

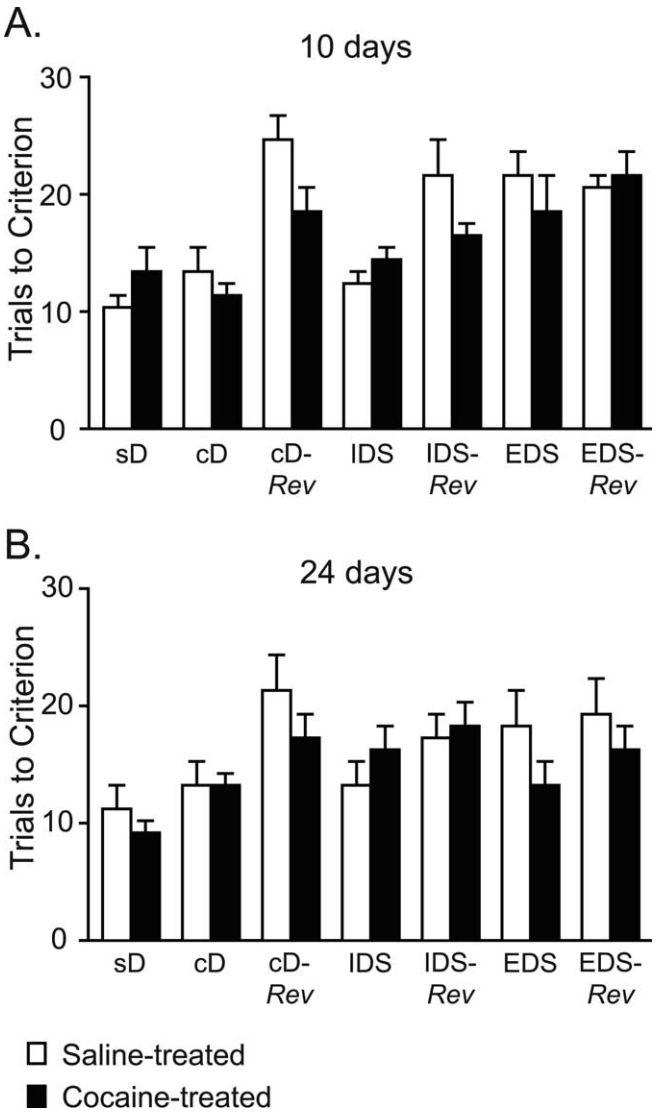

Figure 4. Trials to reach criterion performance for all phases of the ASST in rats treated during adolescence with cocaine and saline. ***A***, Performance 10 d after treatment (cocaine, $n = 12$; saline, $n = 11$). ***B***, Performance 24 d after treatment (cocaine, $n = 11$; saline, $n = 10$). Values represent the mean + SEM. The criterion was as follows: six consecutive trials correct.

Experiment 3: Effects of treatment with cocaine during adolescence on gene expression in the medial PFC

Gene array analysis 22 h after the last cocaine injection showed 10,879 transcripts expressed above detection threshold in 50% or more of the samples. Of these, 201 showed a significant difference with a value of $p \leq 0.05$. Of the 201 significantly regulated genes, 145 were upregulated. The upregulated genes fell into the gene ontology (GO) categories of "cell adhesion" and "extracellular matrix," and the downregulated genes fell into the GO categories of "actin cytoskeleton" and "transcription factor activity" (Table 2). Twenty-four days after discontinuation of cocaine treatment, 63 genes were differently regulated ($p \leq 0.05$), with 22 of these genes upregulated. These 63 genes did not fall into specific GO categories. Only four transcripts were regulated at both time points (22 h and 24 d after cocaine), one of them, dual-specificity phosphatase 6 (MAP kinase phosphatase 3; locus link 116663), was downregulated at both time points, whereas one, D11lgp1 (protein identity predicted through sequence similarities with human and mouse genes; locus link 303512), was upregulated. The two remaining transcripts were ESTs, upregulated 22 h and downregulated 24 d after discontinuation of cocaine treatment. Because a large majority of genes regulated by cocaine treatment fell into the cell adhesion category, three genes were chosen for verification with Q-PCR. Cell adhesion genes aggrecan 1 (locus link 58968), integrin α 6 (locus link 114517), and myosin heavy polypeptide 3 (locus link 24583) were significantly upregulated in the medial PFC 22 h after cocaine treatment (aggrecan, $t_{10} = 2.23$, $p = 0.04$; integrin, $t_9 = 2.76$, $p = 0.02$; myosin, $t_{10} = 2.36$, $p = 0.03$).

Experiment 4: Effects of treatment with cocaine during adolescence on histone modification in the medial PFC

The trimethylated histone protein bands were detected at ~17 kDa, and actin bands were detected at 40 kDa (Fig. 5*A*). Data for the histone proteins were normalized to actin, which was not changed between cocaine- and saline-treated groups either in the immunoblots or in the gene array analyses.

Treatment with cocaine during adolescence significantly reduced the levels of Lys^4-trimethlyated-H3 ($t_{12} = -2.67$; $p = 0.02$) and Lys^{27}-trimethlyated-H3 ($t_{12} = -3.10$; $p = 0.009$) in the medial PFC of rats killed 22 h after their final injection of cocaine, relative to saline (Fig. 5*B*). Both methylation sites are correlated with chromatin activity, and it has been shown that Lys^4 methylation is enriched in the active regions of chromatin (Strahl et al., 1999), whereas Lys^{27} methylation is associated with inactivation of the X chromosome (Plath et al., 2003).

Discussion

Effects of early developmental exposure to cocaine on behavior

Adult rats (P70) treated with binge cocaine during adolescence showed highly sensitized responses to cocaine 24 d after treatment. Sensitization was not, however, observed in younger rats (P56) tested 10 d after treatment. The difference in sensitization between P56 and P70 rats might be a consequence of the time elapsed between cocaine exposure and sensitization testing, because sensitization increases with longer withdrawal periods (Hooks et al., 1994; Brandon et al., 2001), although a contribution of age cannot be excluded. Of interest, the group of rats performing the ASST showed an analogous pattern of altered behavior (i.e., a difference in response between P56 and P70 rats treated with cocaine during adolescence). When presented with a new discrimination problem in which the reinforced dimension changed (EDS) (e.g., odor to medium), control P56 and P70 rats had difficulty shifting attention away from the previous attentional set, an expected outcome that is similar to performance in normal humans (Owen et al., 1991) and monkeys (Dias et al., 1997). Surprisingly, P70 rats treated with cocaine during adolescence shifted their attention rapidly between stimulus dimensions, a phenomenon not observed in cocaine-treated P56 rats.

The behavioral changes may be caused by cocaine-induced morphological and/or neurochemical alterations. For example, increased dendritic spine density and branching in the PFC after chronic administration of cocaine (Robinson and Kolb, 1999) might influence behavior in the ASST. Similar adaptations in the nucleus accumbens have been functionally linked to the expression of behavioral sensitization (Li et al., 2004). Moreover, early cocaine exposure may induce long-term changes in dopamine neurotransmission that affect attentional processing in adulthood. Sensitization, as observed in this study 24 d after adolescent cocaine treatment, is accompanied by augmented responsiveness to dopamine in the PFC (Williams and Steketee, 2005). Because

Table 2. Groups of genes altered in the medial PFC 22 h after the last cocaine injection

Probe set	Gene	Locus link	Fold change natural	*p* value $\log_2$	*p* call %
Downregulated: 38 annotated genes					
Transcription					
1368321 at	EGR 1	24330	−1.43	0.002669	100
1387306 a at	EGR 2	114090	−1.8	0.04767	100
1368650 at	TGFB inducible EGR	81813	−1.31	0.003888	100
1370454 at	homer 1	29546	−2.05	0.000879	100
1370997 at	homer 1	29546	−1.74	0.004621	100
1398362 at	notch gene homolog 2	29492	−1.13	0.037073	100
1369276 at	SMAD 5	59328	−1.06	0.034873	91.5
1369007 at	nuclear receptor subfamily 4, group A, member 2	54278	−1.29	0.012818	100
1370510 a at	aryl hydrocarbon receptor nuclear translocator-like	29657	−1.24	0.004746	100
1376125 at	Zinc finger and BTB domain containing 10	80338	−1.24	0.00269	100
1370975 at	jumonji domain containing 1A	312440	−1.32	0.019277	100
Actin cytoskeleton					
1373849 at	similar to BAF53a	361925	−1.04	0.042658	91.5
1369358 a at	huntingtin-associated protein 1	29430	−1.12	0.042675	100
1370890 at	ARP3 actin-related protein 3	81732	−1.07	0.031892	100
Upregulated: 92 annotated					
Extracellular matrix, cell adhesion					
1367922 at	ADAM 17	57027	1.17	0.006728	100
1387355 at	aggrecan 1	58968	1.09	0.034571	66
1368379 at	CD36 antigen (collagen type I receptor)-like 2	117106	1.32	0.044824	100
1368290 at	cysteine rich protein 61	83476	1.19	0.015106	83
1373947 at	dermatopontin	289178	1.12	0.042523	100
1388111 at	elastin	25043	1.13	0.03281	50
1368187 at	glycoprotein nmb	113955	1.21	0.014259	74.5
1371185 at	integrin, alpha 6	114517	1.18	0.001105	100
1389306 at	matrilin 2	299996	1.14	0.000901	100
1375653 at	neurexin 3	116508	1.12	0.01097	50
1388950 at	procollagen, type IX, alpha 2	362584	1.26	0.043244	58
1389666 at	rod outer segment membrane protein 1	309201	1.12	0.021573	100
1387326 at	sperm adhesion molecule	117037	1.08	0.015186	66.5
1388145 at	tenascin XA	25602	1.13	0.010238	91.5
1370940 at	tight junction protein 2	115769	1.15	0.017142	100
1390292 at	transmembrane protein 8	303004	1.09	0.034561	100
Microfilament motor activity					
1368415 at	myosin, heavy 3	24583	1.21	0.003893	50
1398248 s at	myosin heavy, 6; myosin, heavy 7	29556	1.16	0.030431	50
1369901 at	tubulin, beta 3	246118	1.09	0.030065	74.5

Of 56 downregulated genes, 34 were annotated in databases (i.e., of known function). Of these 34 genes, 10 coded for transcription factors ($p \leq 0.003$ for transcription; GO database), and 3 were related to the actin cytoskeleton ($p \leq 0.005$ for actin cytoskeleton; GO database). Of 145 upregulated genes, 92 were annotated. Sixteen of the annotated genes coded for extracellular matrix or cell adhesion molecules ($p \leq 0.001$ for cell adhesion; GO database), and 3 coded for microfilament motor activity ($p \leq 0.005$; GO database). For datasets, see http://www.mclean.harvard.edu/research/mrc/npl.php.

attention depends on dopamine levels in the PFC (Arnsten and Goldman-Rakic, 1998), altered responsiveness to dopamine could affect ASST performance.

One interpretation of these data are that early cocaine exposure leads to an impairment in forming strong attentional sets, which when formed normally should result in some delay shifting attention from a reinforced attentional set to a previously irrelevant (ignored) stimulus dimension (Isaacs and Duncan, 1962; Mackintosh, 1965). Weakened set formation may be caused by either unusually low levels of perseveration, or a deficit in "learned irrelevance," defined as the delayed ability to focus attention on a previously irrelevant stimulus (Baker and Mackintosh, 1977). Impairments in either condition would result in rapid attentional shifts.

Another interpretation is that early cocaine exposure enhances set shifting in adulthood. Of the few studies that report rapid EDS acquisition, performance was linked to acute changes in dopamine levels in the PFC and striatum (Roberts et al., 1994; Crofts et al., 2001; Tunbridge et al., 2004). As such, if PFC responsiveness to dopamine is augmented after long-term withdrawal from cocaine (Williams and Steketee, 2005), improvements in performance on a dopamine-involved attentional task might be observed (Floresco et al., 2006), because increases in dopamine in the PFC have been previously shown to enhance performance on working memory and attentional tasks (Cai and Arnsten, 1997; Granon et al., 2000).

Under different conditions, either rapid attentional shifts or failures to properly attend to stimuli could be interpreted as attention deficits. Indeed, the PFC and the associated dopamine system are thought to play a role in attention deficit–hyperactivity disorder (Sullivan and Brake, 2003). The ASST was used here as a tool to assess the long-term consequences of cocaine exposure during adolescence on PFC-mediated behaviors in rats. As such, this study demonstrates that PFC function is altered far beyond the period of cocaine exposure. Although it may be difficult to qualify these attentional alterations as impairment or improvement, it is important to consider that the PFC in humans is involved in a large range of different functions, including work-

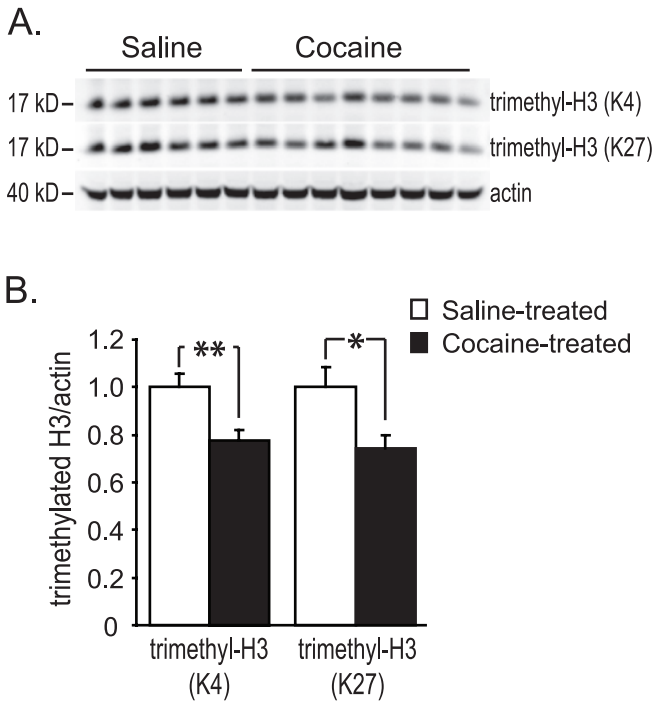

Figure 5. Western blot analysis of two methylation sites of histone H3 in medial PFC tissue of rats 22 h after cocaine treatment (P47). ***A***, Immunoblot analysis with antibodies against trimethylated (Lys^4) (K4) histone H3 and trimethylated (Lys^{27}) (K27) histone H3. Actin was used as a loading control. ***B***, Average levels of both modified histones corrected for loading errors. Values represent the mean + SEM of six saline- and eight cocaine-treated animals. $*p \leq 0.05$; $**p \leq 0.01$.

ing memory, action planning, response inhibition, decision-making, reward processes, and social behavior (Miller and Cohen, 2001; Ridderinkhof et al., 2004). Any lasting impact cocaine has on these functions could be detrimental, particularly in adolescents.

Effects of early developmental exposure to cocaine on gene expression in the PFC

Little is known about long-term physiological adaptations in the medial PFC after cocaine exposure during adolescence, although it is hypothesized that molecular mechanisms are responsible for the enduring behavioral effects associated with cocaine addiction (Nestler, 2004). To examine the molecular consequences of early cocaine exposure in the PFC, we analyzed medial PFC gene expression patterns 22 h and 24 d after binge cocaine administration. Because gene array experiments yield a large volume of data, an analysis focused on broad biological themes, rather than on specific genes (Konradi, 2005), is more robust and informative. Thus, we focused on patterns of gene expression.

Twenty-two hours after the last cocaine injection, we found increased expression of transcripts involved in cell adhesion and microfilament motor activity, and decreased expression of transcription factor genes and transcripts coding for proteins binding to the actin cytoskeleton. These findings raise the possibility that increased synthesis of cell adhesion proteins impede synaptic plasticity by leading to the random formation or strengthening of synapses, rather than organized synapse formation involved in information processing. Enhanced cell adhesion might make these synapses less adaptable and impede the proper formation and reinforcement of learning-specific connections. Previous research has reported upregulations in genes associated with cell adhesion within the nucleus accumbens (Brenz Verca et al., 2001; Freeman et al., 2001) and ventral tegmental area, with links to behaviors such as sensitization (Michna et al., 2001; Bahi et al., 2004).

A smaller number of transcripts involved in microfilament motor activity was upregulated. The proteins encoded by these transcripts are constituents of microtubules (tubulin) and motor proteins (myosin) involved in actin-dependent transport along axons. A different subgroup of actin-binding proteins was downregulated, however, suggesting that cocaine has a complex effect on the actin cytoskeleton and its associated factors (Toda et al., 2006). These results, considered with the present findings, suggest potential synaptic rearrangements in response to cocaine treatment.

Decreased expression of transcription factor genes 22 h after cocaine treatment is another important change identified in these studies. This result is in line with previous research on immediate early transcription factor genes, which peak immediately after cocaine exposure and fall below baseline levels thereafter, particularly after repeated cocaine challenge (Ennulat et al., 1994). The altered expression of the group of transcription factors found in the present gene array study would likely have a significant impact on gene expression patterns, one mechanism by which drugs of abuse can induce long-lasting changes in the brain (Hope, 1996; Nestler, 1997).

Twenty-four days after cocaine treatment, a substantially smaller number of genes showed altered expression levels, and they did not fall into specific categories. Thus, most of the gene expression changes induced by cocaine were transient. However, if early cocaine exposure triggered changes in cell structure/adhesion, the impact of those alterations could be long-lasting. This view is supported by a study using the psychostimulant methylphenidate, which indicated that short-term gene expression changes led to enduring functional changes in striatal circuits (Adriani et al., 2005).

Effects of early developmental exposure to cocaine on histone modification in the PFC

Epigenetic factors can lead to the activation or repression of gene expression through chromatin remodeling, which is mediated by modification of histone proteins. Chromatin remodeling is an important regulatory mechanism for cocaine-induced neural and behavioral plasticity in the striatum (Kumar et al., 2005). We showed that, in the PFC, cocaine induced demethylation of histone H3 at two different sites. Both methylation sites are correlated with chromatin activity; methylation of H3 (K4) is associated with transcriptional activation, whereas methylation of H3 (K27) is associated with transcriptional silencing (Turner, 2002; Sims et al., 2003). The observed methylation may take place in combination with other histone modifications, such as acetylation and phosphorylation, which might further affect transcription. Although a direct connection was not made between the cocaine-induced demethylation of histones in the PFC and the observed changes in PFC gene expression, the ability of cocaine to modify histone proteins could be an epigenetic mechanism by which mRNA transcript levels are altered in the medial PFC.

In conclusion, we demonstrate that binge cocaine administration during adolescence has long-lasting effects on PFC-mediated behavior. We also show that cocaine alters gene expression patterns and histone modification in the PFC. These neuroadaptations could have serious implications, particularly in the developing brain. Although a causal relationship between these cocaine-induced molecular and behavioral adaptations can

only be inferred at this time, it is clear that cocaine exposure during adolescence has far-reaching molecular and behavioral consequences in the rat PFC that develop over time and endure long after drug administration has ceased.

References

Adriani W, Leo D, Greco D, Rea M, di Porzio U, Laviola G, Perrone-Capano C (2006) Methylphenidate administration to adolescent rats determines plastic changes on reward-related behavior and striatal gene expression. Neuropsychopharmacology 31:1946–1956.

Andersen SL (2003) Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci Biobehav Rev 27:3–18.

Andersen SL, Arvanitogiannis A, Pliakas AM, LeBlanc C, Carlezon Jr WA (2002) Altered responsiveness to cocaine in rats exposed to methylphenidate during development. Nat Neurosci 5:13–14.

Arnsten AF, Goldman-Rakic PS (1998) Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. Arch Gen Psychiatry 55:362–368.

Bahi A, Boyer F, Kafri T, Dreyer JL (2004) CD81-induced behavioural changes during chronic cocaine administration: in vivo gene delivery with regulatable lentivirus. Eur J Neurosci 19:1621–1633.

Baker AG, Mackintosh NJ (1977) Excitatory and inhibitory conditioning following uncorrelated presentations of CS and UCS. Anim Learn Behav 5:315–319.

Bechara A, Dolan S, Denburg N, Hindes A, Anderson SW, Nathan PE (2001) Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. Neuropsychologia 39:376–389.

Berger SL (2002) Histone modifications in transcriptional regulation. Curr Opin Genet Dev 12:142–148.

Birrell JM, Brown VJ (2000) Medial frontal cortex mediates perceptual attentional set shifting in the rat. J Neurosci 20:4320–4324.

Bolanos CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ (2003) Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. Biol Psychiatry 54:1317–1329.

Bolla KI, Rothman R, Cadet JL (1999) Dose-related neurobehavioral effects of chronic cocaine use. J Neuropsychiatry Clin Neurosci 11:361–369.

Bolla KI, Eldreth DA, London ED, Kiehl KA, Mouratidis M, Contoreggi C, Matochik JA, Kurian V, Cadet JL, Kimes AS, Funderburk FR, Ernst M (2003) Orbitofrontal cortex dysfunction in abstinent cocaine abusers performing a decision-making task. NeuroImage 19:1085–1094.

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19:185–193.

Brandon CL, Marinelli M, Baker LK, White FJ (2001) Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. Neuropsychopharmacology 25:651–661.

Brenz Verca MS, Widmer DA, Wagner GC, Dreyer J (2001) Cocaine-induced expression of the tetraspanin CD81 and its relation to hypothalamic function. Mol Cell Neurosci 17:303–316.

Cai J, Arnsten AF (1997) Dose-dependent effects of the dopamine D1 receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. J Pharmacol Exp Ther 283:183–189.

Carlezon Jr WA, Konradi C (2004) Understanding the neurobiological consequences of early exposure to psychotropic drugs: linking behavior with molecules. Neuropharmacology 47:47–60.

Casey BJ, Giedd JN, Thomas KM (2000) Structural and functional brain development and its relation to cognitive development. Biol Psychol 54:241–257.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156:11–18.

Cole R, Konradi C, Douglass J, Hyman S (1995) Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. Neuron 14:813–823.

Crofts HS, Dalley JW, Collins P, Van Denderen JC, Everitt BJ, Robbins TW, Roberts AC (2001) Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. Cereb Cortex 11:1015–1026.

Dahlquist KD, Salomonis N, Vranizan K, Lawlor SC, Conklin BR (2002) GenMAPP, a new tool for viewing and analyzing microarray data on biological pathways. Nat Genet 31:19–20.

Dias R, Robbins TW, Roberts AC (1997) Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from "on-line" processing. J Neurosci 17:9285–9297.

Ennulat DJ, Babb S, Cohen BM (1994) Persistent reduction of immediate early gene mRNA in rat forebrain following single or multiple doses of cocaine. Brain Res Mol Brain Res 26:106–112.

Floresco SB, Magyar O, Ghods-Sharifi S, Vexelman C, Tse MT (2006) Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. Neuropsychopharmacology 31:297–309.

Freeman WM, Nader MA, Nader SH, Robertson DJ, Gioia L, Mitchell SM, Daunais JB, Porrino LJ, Friedman DP, Vrana KE (2001) Chronic cocaine-mediated changes in non-human primate nucleus accumbens gene expression. J Neurochem 77:542–549.

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642–1652.

Goussakov I, Chartoff EH, Tsvetkov E, Gerety LP, Meloni EG, Carlezon Jr WA, Bolshakov VY (2006) LTP in the lateral amygdala during cocaine withdrawal. Eur J Neurosci 23:239–250.

Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW (2000) Enhanced and impaired attentional performance after infusion of D_1 dopaminergic receptor agents into rat prefrontal cortex. J Neurosci 20:1208–1215.

Hooks MS, Duffy P, Striplin C, Kalivas PW (1994) Behavioral and neurochemical sensitization following cocaine self-administration. Psychopharmacology (Berl) 115:265–272.

Hope BT (1996) Novel transcription factors are induced by chronic cocaine treatment. Ann NY Acad Sci 801:1–12.

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4:249–264.

Isaacs ID, Duncan CP (1962) Reversal and nonreversal shifts within and between dimensions in concept formation. J Exp Psychol 64:580–585.

Konradi C (2005) Gene expression microarray studies in polygenic psychiatric disorders: Applications and data analysis. Brain Res Brain Res Rev 50:142–155.

Konradi C, Cole R, Heckers S, Hyman S (1994) Amphetamine regulates gene expression in rat striatum via transcription factor CREB. J Neurosci 14:5623–5634.

Konradi C, Leveque J-C, Hyman S (1996) Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. J Neurosci 16:4231–4239.

Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, Neve RL, Self DW, Nestler EJ (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303–314.

Li C, Wong WH (2001) Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. Proc Natl Acad Sci USA 98:31–36.

Li Y, Acerbo MJ, Robinson TE (2004) The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. Eur J Neurosci 20:1647–1654.

Mackintosh NJ (1965) Selective attention in animal discrimination learning. Psychol Bull 64:124–150.

McAlonan K, Brown VJ (2003) Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. Behav Brain Res 146:97–103.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 21:8655–8663.

McGregor A, Baker G, Roberts DC (1996) Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on intravenous cocaine self-administration under a progressive ratio schedule of reinforcement. Pharmacol Biochem Behav 53:5–9.

Merline AC, O'Malley PM, Schulenberg JE, Bachman JG, Johnston LD (2004) Substance use among adults 35 years of age: prevalence, adulthood predictors, and impact of adolescent substance use. Am J Public Health 94:96–102.

Michna L, Brenz Verca MS, Widmer DA, Chen S, Lee J, Rogove J, Zhou R, Tsitsikov E, Miescher GC, Dreyer JL, Wagner GC (2001) Altered sensi-

tivity of CD81-deficient mice to neurobehavioral effects of cocaine. Brain Res Mol Brain Res 90:68–74.
Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167–202.
Nestler EJ (1997) Molecular mechanisms of opiate and cocaine addiction. Curr Opin Neurobiol 7:713–719.
Nestler EJ (2004) Molecular mechanisms of drug addiction. Neuropharmacology 47 [Suppl 1]:24–32.
O'Malley PM, Johnston LD, Bachman JG (1985) Cocaine use among American adolescents and young adults. NIDA Res Monographs 61:50–75.
Owen AM, Roberts AC, Polkey CE, Sahakian BJ, Robbins TW (1991) Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. Neuropsychologia 29:993–1006.
Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, Ed 4. San Diego: Academic.
Piazza PV, Rouge-Pont F, Deminiere JM, Kharoubi M, Le Moal M, Simon H (1991) Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. Brain Res 567:169–174.
Pierce RC, Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Brain Res Rev 25:192–216.
Plath K, Fang J, Mlynarczyk-Evans SK, Cao R, Worringer KA, Wang H, de la Cruz CC, Otte AP, Panning B, Zhang Y (2003) Role of histone H3 lysine 27 methylation in X inactivation. Science 300:131–135.
Ridderinkhof KR, van den Wildenberg WP, Segalowitz SJ, Carter CS (2004) Neurocognitive mechanisms of cognitive control: the role of prefrontal cortex in action selection, response inhibition, performance monitoring, and reward-based learning. Brain Cogn 56:129–140.
Roberts AC, De Salvia MA, Wilkinson LS, Collins P, Muir JL, Everitt BJ, Robbins TW (1994) 6-Hydroxydopamine lesions of the prefrontal cortex in monkeys enhance performance on an analog of the Wisconsin Card Sort Test: possible interactions with subcortical dopamine. J Neurosci 14:2531–2544.
Robinson TE, Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598–1604.
Santucci AC, Capodilupo S, Bernstein J, Gomez-Ramirez M, Milefsky R, Mitchell H (2004) Cocaine in adolescent rats produces residual memory impairments that are reversible with time. Neurotoxicol Teratol 26:651–661.
Sims III RJ, Nishioka K, Reinberg D (2003) Histone lysine methylation: a signature for chromatin function. Trends Genet 19:629–639.
Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417–463.
Strahl BD, Ohba R, Cook RG, Allis CD (1999) Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in *Tetrahymena*. Proc Natl Acad Sci USA 96:14967–14972.
Sullivan RM, Brake WG (2003) What the rodent prefrontal cortex can teach us about attention-deficit/hyperactivity disorder: the critical role of early developmental events on prefrontal function. Behav Brain Res 146:43–55.
Toda S, Shen HW, Peters J, Cagle S, Kalivas PW (2006) Cocaine increases actin cycling: effects in the reinstatement model of drug seeking. J Neurosci 26:1579–1587.
Tunbridge EM, Bannerman DM, Sharp T, Harrison PJ (2004) Catechol-*o*-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. J Neurosci 24:5331–5335.
Turner BM (2002) Cellular memory and the histone code. Cell 111:285–291.
Unterwald EM, Ho A, Rubenfeld JM, Kreek MJ (1994) Time course of the development of behavioral sensitization and dopamine receptor up-regulation during binge cocaine administration. J Pharmacol Exp Ther 270:1387–1396.
van Leeuwen F, Gottschling DE (2002) Genome-wide histone modifications: gaining specificity by preventing promiscuity. Curr Opin Cell Biol 14:756–762.
Weissenborn R, Robbins TW, Everitt BJ (1997) Effects of medial prefrontal or anterior cingulate cortex lesions on responding for cocaine under fixed-ratio and second-order schedules of reinforcement in rats. Psychopharmacology 134:242–257.
Williams JM, Steketee JD (2005) Time-dependent effects of repeated cocaine administration on dopamine transmission in the medial prefrontal cortex. Neuropharmacology 48:51–61.
Wolf ME, Sun X, Mangiavacchi S, Chao SZ (2004) Psychomotor stimulants and neuronal plasticity. Neuropharmacology 47 [Suppl 1]:61–79.
Wu J, Grunstein M (2000) 25 years after the nucleosome model: chromatin modifications. Trends Biochem Sci 25:619–623.