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Induction and Blockade of Adolescent Cocaine-Induced Habits

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ABSTRACT

BACKGROUND: Cocaine use during adolescence increases vulnerability to drug dependence and decreases the likelihood that individuals will seek treatment as adults. Understanding how early-life cocaine exposure influences decision-making processes in adulthood is thus critically important.

METHODS: Adolescent or adult mice were exposed to subchronic cocaine, then behavioral sensitivity to changes in the predictive relationship between actions and their consequences was tested. Dendritic spines on the principal pyramidal neurons of the orbitofrontal prefrontal cortex (oPFC) were also imaged and enumerated. To determine whether cytoskeletal regulatory systems in the oPFC influenced decision-making strategies, we then inhibited the activity of Abl family and Rho kinases as well as NR2B-containing *N*-methyl-D-aspartate receptors. We also attempted to block the reinstatement of cocaine seeking in cocaine self-administering mice.

RESULTS: Adult mice with a history of subchronic cocaine exposure in adolescence engaged habit-based response strategies at the expense of goal-directed decision-making strategies and had fewer dendritic spines in the oPFC. Inhibition of the cytoskeletal regulatory Abl family kinases in the oPFC recapitulated these neurobehavioral deficiencies, whereas Rho kinase inhibition corrected response strategies. Additionally, the NR2B-selective *N*-methyl-D-aspartate receptor antagonists ifenprodil and CP-101,606 blocked cocaine-induced habits; this was dependent on Abl family signaling in the oPFC. Ifenprodil also mitigated cue-induced reinstatement of cocaine seeking in mice self-administering cocaine.

CONCLUSIONS: We suggest that adolescent cocaine exposure confers a bias toward habit-based behavior in adulthood via long-term cellular structural modifications in the oPFC. Treatments aimed at mitigating the durable consequences of early-life cocaine use may benefit from targeting cytoskeletal regulatory systems.

Keywords: Abl2, Arg kinase, Ifenprodil, Incubation, OFC, Orbital, Response-outcome

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Adolescent-onset cocaine abuse, relative to adult-onset abuse, increases vulnerability to developing drug dependence and decreases the likelihood that individuals will seek treatment across the life span (1,2). Understanding how early-life cocaine exposure influences decision-making processes may inform novel treatment approaches, yet neurobiological research into the long-term effects of adolescent drug exposure is limited.

"Incubation" typically refers to progressive, time-dependent enhancements in drug craving and is thought to contribute to the maintenance and persistence of addiction (3,4). Progressive drug-induced modifications in decision-making processes may additionally contribute to addiction etiology, however (5,6). For example, a bias toward engaging stimulus-response habits, at the expense of goal-oriented behavioral response strategies, is considered an etiological factor in addiction (5,7). Accordingly, several groups have reported that a history of chronic cocaine or amphetamine exposure induces "reward-seeking" habits in adult rodents (8). Additionally, drug-induced deficits in reversal tasks appear to incubate in tandem with cocaine-seeking behaviors (9).

We trained mice to generate two distinct instrumental responses, and then we decreased the likelihood that one familiar response would be reinforced. "Goal-directed" rodents will inhibit the behavior that is no longer likely to be rewarded, interpreted as evidence of knowledge of the response-outcome relationship (10). Conversely, equivalent engagement of both responses can reflect a habit-based deferral to familiar response patterns. This may occur as a result of extensive response training (10) or a failure to learn, retain, or use new response-outcome associations. We find that subchronic cocaine exposure in adolescence, but not adulthood, causes failures in response-outcome conditioning, inducing inflexible, habit-like behavior. This phenomenon incubates, emerging in adulthood.

The orbitofrontal prefrontal cortex (oPFC) becomes progressively hypoactive during prolonged cocaine withdrawal in humans (11) and is essential to selecting actions based on their outcomes (12,13). Adolescent cocaine exposure in our experiments reduced dendritic spine density in the adult oPFC. Meanwhile, inhibition of Abl family kinases, cytoskeletal regulatory factors highly expressed in the PFC, recapitulated

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the effects of cocaine. These findings served as a platform from which to develop intervention strategies to enhance new learning regarding response-outcome relationships in cocaine-exposed mice. We suggest that treatments for individuals first exposed to cocaine as adolescents [by some estimates, 90% of cocaine users (14)] might benefit from targeting learning and memory systems regulated by cytoskeletal signaling factors, rather than the reinforcing properties of the drug.

METHODS AND MATERIALS

Subjects

Male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) or transgenic mice expressing *thy1*-driven yellow fluorescent protein (15) and back-crossed onto a C57BL/6 background were used. Mice were maintained on a 12-hour light/dark cycle (8 AM on), experimentally naïve, and provided food and water ad libitum unless otherwise indicated. Procedures were approved by the Emory University Institutional Animal Care and Use Committee.

Experimenter-Administered Cocaine or Amphetamine

Cocaine (10 mg/kg), D-amphetamine (3 mg/kg), or saline was administered for 5 consecutive days (intraperitoneal, 1 mL/100 g; Sigma-Aldrich, St. Louis, MO). Mice were then left undisturbed until instrumental response training. Table 1 shows the timing of injections and training.

Instrumental Response Training

Adult mice (≥ postnatal day [P] 56) were food-restricted to approximately 90% of their free-feeding body weight. When adolescent (P36) mice were tested, feeding was merely titrated to allow for typical weight gain according to Jackson Laboratory growth trajectories.

Mice were trained to nose poke for food reinforcers (20 mg grain-based Bio-Serv pellets [Bio-Serv, Flemington, NJ]) in Med Associates operant conditioning chambers (Med Associates, Inc., St. Albans, VT) equipped with two nose poke recesses and a food magazine. Responding was reinforced using a fixed ratio 1 (FR1) schedule of reinforcement in which 30 pellets were available for responding on the two distinct nose poke recesses, resulting in 60 pellets/session (five sessions). The sessions ended at 135 minutes or when mice acquired all 60 pellets. In our final experiments (using coadministered ifenprodil and STI-571, extended training, or CP-101,606), sessions were shortened to 70 minutes for expediency. For extended training experiments, mice were reinforced according to a random interval 30-second schedule for two sessions after FR1 training. Then a random interval 60-second schedule was used for three additional sessions.

Response-Outcome Contingency Degradation

A modified version of response-outcome contingency degradation was used, as in our prior reports (13,16,17) and similar to Barker *et al.* (18): in a 25-minute "non-degraded" session, one nose poke aperture was occluded, and responding on the other aperture was reinforced using an FR1 schedule of reinforcement, as during training. In the 25-minute "degraded" session, the opposite aperture was occluded, and reinforcers were delivered into the magazine at a rate matched to each animal's reinforcement rate from the previous session. Under these conditions, only approximately 7% of pellets are delivered (by chance) within 2 seconds after a response (19). Thus, this response becomes significantly less predictive of reinforcement than the other response. These sessions, and which response-outcome contingency was degraded, were counterbalanced.

The following day, both apertures were available during a 10-minute probe test conducted in extinction. A goal-directed response strategy is to preferentially engage the response that is likely to be reinforced, whereas a habit-based strategy is to engage both familiar responses equivalently, irrespective of the likelihood of reinforcement (10). In one experiment, mice were then retrained to nose poke on both apertures using an FR1 schedule of reinforcement for five additional sessions, and the procedure was repeated.

Fasudil, Ifenprodil, and CP-101,606

Mice were administered fasudil (10 mg/kg, in saline; LC Laboratories, Woburn, MA); ifenprodil (10 mg/kg, in water; Tocris Bioscience, Avonmouth, Bristol, United Kingdom); CP-101,606 (3 mg/kg, in 20% dimethylsulfoxide and water; Sigma-Aldrich); or the corresponding vehicle (intraperitoneal, 1 mL/100 g). Doses were determined based on previous work (17,20–22). CP-101,606 dosing was also determined based on an attempt to match affinity for the *N*-methyl-D-aspartate receptor (NMDAR) NR2B subunit to the selected dose of ifenprodil, a less specific antagonist (23). The timing of injections is described in Table 1 and throughout Results.

Dendritic Spine Imaging and Quantification, Intracranial Infusions, Histology, Cocaine Self-administration, and Cardiovascular Assessments

Dendritic spine imaging and quantification, intracranial infusions, histology, cocaine self-administration, and cardiovascular assessments were performed as described elsewhere (19,20) and in Supplemental Methods and Materials.

Statistical Analyses

Response rates, dendritic spine measures, and cardiovascular metrics were compared by *t* test or analysis of variance with repeated measures and Tukey's post hoc comparisons when appropriate. When spine densities were normalized to saline control values, one-sample *t* tests against 0 (no change) were also applied. Dendritic spine head diameters were compared by Kolmogorov-Smirnov tests. Values >2 SD above the mean were considered outliers and excluded. A p value $< .05$ was considered significant.

RESULTS

We aimed to identify whether adolescent mice are vulnerable to cocaine-induced impairments in selecting actions based on their consequences. An overview of experiments is presented in Table 1. Mice were first exposed to cocaine from P31 to P35, early adolescence (20,24,25), given that early-adolescent cocaine use greatly increases the risk of later drug dependence