Induction and Blockade of Adolescent Cocaine-Induced Habits

Lauren M. Depoy, *Emory University*
Kelsey S. Zimmermann, *Emory University*
Paul J. Marvar, *George Washington University*
Shannon L Gourley, *Emory University*

Journal Title: Biological Psychiatry
Volume: Volume 81, Number 7
Publisher: Elsevier: 12 months | 2017-04-01, Pages 595-605
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.biopsych.2016.09.023
Permanent URL: https://pid.emory.edu/ark:/25593/s8xr5

Final published version: http://dx.doi.org/10.1016/j.biopsych.2016.09.023

HHS Public Access

Author manuscript

Biol Psychiatry. Author manuscript; available in PMC 2018 April 01.

Published in final edited form as:

Biol Psychiatry. 2017 April 01; 81(7): 595–605. doi:10.1016/j.biopsych.2016.09.023.

Induction and blockade of adolescent cocaine-induced habits

Lauren M. DePoy[1,2], **Kelsey S. Zimmermann**[1,2], **Paul J. Marvar**[3], and **Shannon L. Gourley**[1,2,*]

[1]Departments of Pediatrics and Psychiatry, Emory University School of Medicine

[2]Yerkes National Primate Research Center, Graduate Program in Neuroscience, Emory University

[3]Department of Pharmacology and Physiology, Department of Psychiatry and Behavioral Sciences, GW Institute for Neuroscience, The George Washington University

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 NMDA 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, while Rho-kinase inhibition corrected response strategies. Additionally, the NR2B-selective NMDA receptor antagonists ifenprodil and CP-101,606 blocked cocaine-induced habits, and 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 towards habit-based behavior in adulthood via long-term cellular structural modifications in the oPFC. Treatments

*Contact: Shannon L. Gourley, PhD, Department of Pediatrics | Graduate Program in Neuroscience, Yerkes National Primate Research Center, Emory University, 954 Gatewood Rd. NE, Atlanta GA 30329, 404-727-2482, shannon.l.gourley@emory.edu.

Author Contributions: All authors conducted experiments. LMD and SLG analyzed data and prepared the manuscript.

Financial Disclosures: The authors report no biomedical financial interests or potential conflicts of interest.

aimed at mitigating the durable consequences of early-life cocaine may benefit from targeting cytoskeletal regulatory systems.

Keywords

OFC; orbital; incubation; ifenprodil; response-outcome; Abl2; Arg kinase

Introduction

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 lifespan(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). *E.g.*, a bias towards 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 [reviewed(8)] Additionally, drug-induced deficits in reversal tasks appear to incubate in tandem with cocaine-seeking behaviors(9).

Here we trained mice to generate two distinct instrumental responses, then we decreased the likelihood that one familiar response would be reinforced. Rodents that are “goal-directed” 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 due to extensive response training(10) or a failure to learn, retain, or utilize 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 here reduced dendritic spine density in the adult oPFC. Meanwhile, inhibition of Abl-family kinases, cytoskeletal regulatory factors highly expressed in the PFC, recapitulated 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 the cocaine-using population(14)] might benefit from targeting learning and memory systems regulated by cytoskeletal signaling factors, *e.g.*, rather than the reinforcing properties of the drug.

Methods

Subjects

Male C57BL/6 mice (Jackson Labs) or transgenic mice expressing *thy1*-driven Yellow Fluorescent Protein (YFP)(15) and back-crossed onto a C57BL/6 background were used. Mice were maintained on a 12-hour light cycle (0800 on), experimentally naïve, and provided food and water *ad libitum* unless otherwise indicated. Procedures were Emory University IACUC-approved.

Experimenter-administered cocaine, amphetamine

Cocaine (10 mg/kg), D-amphetamine (3 mg/kg), or saline was administered for 5 consecutive days (Sigma; i.p., 1 ml/100 g). Mice were then left undisturbed until instrumental response training. See table 1 for the timing of injections and training.

Instrumental response training

Adult mice [≥postnatal day (P) 56] were food-restricted to ~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) in Med-Associates operant conditioning chambers equipped with 2 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 2 distinct nose poke recesses, resulting in 60 pellets/session (5 sessions). The sessions ended at 135 min or when mice acquired all 60 pellets. In our final experiments (using co-administered ifenprodil and STI-571, extended training, or CP-101-606), sessions were shortened to 70 min for expediency.

For extended training experiments, mice were reinforced according to a random interval (RI) 30-sec schedule for 2 sessions after FR1 training. Then, an RI-60-sec schedule was used for 3 additional sessions.

Response-outcome contingency degradation

A modified version of response-outcome contingency degradation was used, as in our prior reports (*e.g.*,16,17,13), and similar to(18): In a 25-min “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-min “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 ~7% of pellets are delivered (by chance) within 2 seconds following a response(19). Thus, this response becomes significantly less predictive of reinforcement than the other. These sessions, and which response-outcome contingency was “degraded,” were counter-balanced.

The following day, both apertures were available during a 10-min probe test conducted in extinction. A “goal-directed” response strategy is to preferentially engage the response that is likely to be reinforced, while a habit-based strategy is to engage both familiar responses equivalently, irrespective of the likelihood of reinforcement(10).

In one experiment, mice were then re-trained to nose poke on both apertures using an FR1 schedule of reinforcement for 5 additional sessions, and the procedure was repeated.

Fasudil, ifenprodil, CP-101,606

Mice were administered fasudil (10 mg/kg, in saline; LC labs); ifenprodil (10 mg/kg, in water; Tocris); CP-101,606 (3 mg/kg, in 20% DMSO+water; Sigma); or the corresponding vehicle (i.p., 1 ml/100g). Doses were determined based on(17,20,21,22). CP-101,606 dosing was also determined based on an attempt to match affinity for the NMDA receptor NR2B subunit to the selected dose of ifenprodil, a less specific antagonist(23). The timing of injections is described in table 1 and throughout the Results section.

Dendritic spine imaging and quantification, intracranial infusions, histology, cocaine self-administration, and cardiovascular assessments were performed as described [see(19,20);Supplementary Methods].

Statistical analyses

Response rates, dendritic spine measures, and cardiovascular metrics were compared by *t*-test or ANOVA with repeated measures and Tukey's post-hoc comparisons when appropriate. When spine densities were normalized to saline control values, 1-sample *t*-tests against 0 (no change) were also applied. Dendritic spine head diameters were compared by Kolmogorov-Smirnov (K-S) tests. Values >2 standard deviations above the mean were considered outliers and excluded. $p<0.05$ 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 in table 1. Mice were first exposed to cocaine from P31-35, early adolescence(20,24,25), given that early-adolescent cocaine use greatly increases risk of later drug dependence in humans(26). A group of preadolescent [P24-28(24)] cocaine-exposed mice served as a comparison. Mice were then trained as P56 adults to respond on two distinct nose poke recesses for food reinforcers. Throughout, response acquisition curves represent total responses/minute, and mice acquired the responses with no differences between groups (Fs<1;fig.1a; all others, fig.S1).

An important aspect of goal-directed decision making is selecting between actions that are more, *vs*. less, likely to be reinforced with a desired outcome. Thus, we modified the relationship between one response and the associated outcome by providing the reinforcer associated with that response non-contingently (fig.S2). A goal-oriented response strategy would be to preferentially generate the response that remains reinforced in a subsequent probe test(10). Saline- and P24-28 cocaine-treated mice indeed preferentially performed this

response. Mice exposed to cocaine from P31-35, however, failed to differentiate between the responses [interaction $F_{(2,26)}$=3.8,p=0.04] (fig.1b). Thus, cocaine exposure during early adolescence weakened goal-directed action selection in adulthood.

This experiment was next replicated except mice received cocaine or amphetamine from P31-35. Cocaine-exposed mice again responded indiscriminately [interaction $F_{(2,24)}$=6.8,p=0.02] (fig.1c). Unlike with cocaine, however, amphetamine-exposed mice differentiated between actions that were more, *vs.* less, likely to be reinforced.

Some consequences of cocaine become apparent only after a drug washout period. We thus also ascertained the effects of cocaine when mice were tested *immediately following* adolescent exposure. Interestingly, a main effect of response selection indicated that all mice preferentially generated the response most likely to be reinforced [$F_{(1,16)}$=8.1,p<0.05] (fig. 1d). We next tested the effects of subchronic cocaine exposure in adulthood, from P56-60. One and 3 weeks later, all mice adopted goal-oriented response strategies following instrumental contingency degradation [main effects $F_{(1,16)}$=16.4,p<0.001;$F_{(1,18)}$=20.2,p<0.001] (fig.1e-f). Thus, subchronic cocaine exposure selectively in adolescence impairs goal-directed action selection, and this effect appears to "incubate."

Dendritic spines remodel following adolescent cocaine exposure

Dendritic spines on excitatory neurons in deep-layer oPFC were imaged in adulthood (P56) following subchronic cocaine exposure in early adolescence (P31-35) or preadolescence (P24-28). Spine density was reduced following P31-35 exposure [$F_{(2,21)}$=4,p<0.05] (fig.1g). Further, cocaine at both ages decreased head diameters, suggestive of an immature phenotype (*i.e.*, spines lacking a developed mushroom-like head; both K-S p<0.001)(fig.1h).

We have noted over the course of several unpublished studies that the average length of these oPFC dendritic spines increases during adolescence, as also reported in hippocampal CA1(27). We summarize those findings here [age $F_{(3,77)}$=8.1,p<0.001] (fig.1i). Average spine lengths in adult mice exposed to cocaine in adolescence were, however, shorter relative to other groups [$F_{(2,48)}$=3.9,p<0.03] (fig.1j), an adolescent-like morphology.

Recapitulation and blockade of cocaine-induced habits

Are cellular structural abnormalities in the oPFC associated with failures in response-outcome decision making? We trained naïve P56 mice to respond for food reinforcers (fig.S1), then "degraded" the predictive relationship between one familiar response and its outcome, as above. Immediately following, during the presumptive formation of new memory, we infused into the oPFC saline or STI-571, an Abl-family kinase inhibitor that destabilizes neural structure, *e.g.*, via disinhibition of the RhoA GTPase (Rho)(fig.2a)(28). Subsequently, saline-infused mice preferentially generated the response that was most likely to be reinforced. STI-571-infused mice, by contrast, failed to differentiate between the actions that were more, *vs.* less, likely to be reinforced and instead relied on familiar, habit-like response strategies [interaction $F_{(1,16)}$=11.8,p<0.01] (fig.2b). Thus, Abl-family kinase signaling appears to be essential to consolidating or retaining new information regarding the predictive relationship between actions and their outcomes.

Dendritic spines were imaged and enumerated. As expected, STI-571 reduced dendritic spine densities on excitatory deep-layer oPFC neurons. Spine deficiencies were selective to dendrites within 150 μm of the infusion site, within the anatomical boundaries of the ventrolateral oPFC [interaction $F_{(3,94)}=3.2,p<0.05$] (fig.2c).

We next attempted to *block* the effects of early-life cocaine exposure. We exposed mice to saline or cocaine from P31-35, then trained mice to nose poke as P56 adults as above. We again modified the predictive relationship between a response and its outcome, then administered the Rho-kinase inhibitor fasudil, or the NR2B-selective NMDA receptor (NMDAR) antagonist ifenprodil, immediately following training. Both drugs *amplify* Abl2 (also called Arg) kinase influence (fig.2d)(28,29). As expected, control mice subsequently preferentially generated the response that was most likely to be reinforced, while cocaine-exposed mice treated with vehicle did not differentiate between the two responses. Both fasudil and ifenprodil *induced goal-directed response selection in cocaine-exposed mice* [interaction $F_{(2,54)}=4.6,p=0.01$] (fig.2e). Together, these findings indicate that response-outcome conditioning can be bi-directionally regulated to mimic or block cocaine-induced deficiencies.

Fasudil acts rapidly, with a terminal elimination half-life of <1 hour(30). It is also, at certain doses, a potent vasodilator. To assess cardiovascular function, we administered fasudil to naïve mice bearing indwelling telemeters that measure arterial blood pressure and heart rate. Neither were affected, suggesting that fasudil's actions were not associated with vascular changes (fig.S3).

Effects of ifenprodil in the oPFC

Ifenprodil has reported protective benefits in animal models of alcohol(31), heroin(32), and nicotine(33) relapse. Thus, we focused the rest of our studies on this compound. First, we examined whether acute ifenprodil impacts oPFC dendritic spines. As above, mice were exposed to cocaine from P31-35, tested in a response-outcome contingency degradation task in adulthood, and administered ifenprodil immediately following training. Cocaine exposure reduced dendritic spine density and length as expected, and ifenprodil mitigated both deficiencies [density $F_{(2,100)}=4.5,p=0.013$] [also, 1-sample *t*-test against 0 (no change): for cocaine $t_{(31)}=-2.5,p=0.018$; for ifenprodil+cocaine p=0.27] (fig.2f)(for lengths, see fig.S4). We focused on terminal dendritic branches, considered quite labile and thus potentially sensitive to acute drug treatments, such as ifenprodil here. This experiment thus extends our initial findings that developmental cocaine reduces spine densities within 50-150 μm of the cell soma (fig.1), providing evidence of widespread spine loss.

Next, we assessed whether the habit-blocking effects of ifenprodil were dependent on Abl-family signaling in the oPFC. We exposed mice to cocaine during adolescence, then trained them to respond for food reinforcers. As expected, mice exposed to cocaine in adolescence and then treated with saline developed habit-based behaviors, while ifenprodil restored goal-directed response preference. Simultaneous infusion of the Abl-family kinase inhibitor STI-571 in the oPFC (fig.2g), however, *occluded the effects of ifenprodil*, indicating that the habit-blocking effects of ifenprodil require intact Abl-family signaling in the oPFC [interaction $F_{(1,57)}=4.3,p=0.04$] (fig.2h).

We utilized a 2×2×2 experimental design, leading to the unexpected discovery that mice exposed to cocaine+STI-571 developed goal-directed response preferences (fig.2h). In addition to Abl-family kinases, STI-571 inhibits c-Kit and PDGF receptors(34). These off-target actions may contribute to this unexpected blockade of cocaine-induced habits.

Our findings raise the possibility that ifenprodil could also block habits that develop under more naturalistic circumstances. We extensively trained intact mice to induce habits by virtue of prolonged response training (fig.S1). Ifenprodil was injected immediately following a session when the likelihood of reinforcement was diminished. Subsequently, ifenprodil-treated mice preferentially engaged the response that was most likely to be reinforced, while vehicle-treated mice engaged both responses equally, habitually [interaction $F_{(1,10)}$=10.4,p=0.009] (fig.2i). Thus, ifenprodil can block habit-based behavior induced by either cocaine or extended training.

If ifenprodil is acting on response-outcome memory formation, delayed injection should have no effects since memory consolidation is thought to occur within a narrow time window following a learning event. In a separate group of trained mice, we delayed ifenprodil treatment by 4 hours. In this case, all mice displayed habitual response strategies, suggesting that ifenprodil acts, at least in part, via memory formation processes to enable subsequent response flexibility [effect of response $F_{(1,16)}$=1.7,p=0.2; interaction F<1] (fig. 2j).

Blockade of cocaine-induced inflexibility with CP-101,606

Ifenprodil is highly active at NMDAR NR2B, but it also has off-target effects. Thus, we tested separate mice using the more selective NMDAR NR2B antagonist, CP-101,606.Following response-outcome contingency degradation, control mice preferentially generated the response that was most likely to be reinforced, whereas mice exposed to cocaine in adolescence did not differentiate between two familiar responses, as expected. Like ifenprodil,CP-101,606 *induced goal-directed action in cocaine-exposed mice* [interaction $F_{(1,40)}$=4.8,p<0.05] (fig.3a).

Are cocaine-exposed mice incapable of, or delayed in, developing goal-directed response strategies? We re-trained the same mice for 5 additional sessions using an FR1 schedule of reinforcement that would be expected to bias responding towards outcome-sensitive response strategies. All groups subsequently preferentially generated the response that was most likely to be reinforced following instrumental contingency degradation [response $F_{(1,40)}$=34.1,p<0.001] (fig.3b). Thus, adolescent cocaine exposure impairs response-outcome conditioning, causing a deferral to familiar behavioral patterns, as opposed to accelerating habit formation *per se*.

Ifenprodil reduces cue-induced reinstatement of cocaine seeking

Lastly, we assessed whether pairing ifenprodil with another form of outcome-based conditioning could mitigate relapse-like behavior (fig.4a). Young-adult mice were trained to self-administer cocaine via in-dwelling jugular catheters. A main effect of response indicated that mice responded preferentially on the active *vs.* inactive apertures [$F_{(1,14)}$=9.9,p=0.007], with no differences between mice designated to receive vehicle *vs.*

ifenprodil (F<1)(fig.4b). Mice also required the same amount of training to develop stable response patterns [$t_{(13)}$=-0.7,p=0.5] (fig.4c) and acquired the same number of total cocaine infusions (not shown).

We then extinguished responding by withholding the cocaine reinforcer, and immediately following each training session, injected ifenprodil. Ifenprodil had no effects on response extinction (Fs<1)(fig.4d). Following the re-introduction of cocaine-associated cues, however, ifenprodil-treated mice generated significantly fewer responses on the cocaine-associated aperture [interaction $F_{(1,14)}$=8.8,p=0.01] (fig.4e;S5). Together, these findings suggest that ifenprodil inhibits both cocaine-induced behavioral inflexibility and relapse-like behavior by augmenting response-outcome learning and memory.

Discussion

In cocaine-abusing humans, cocaine craving can progressively increase during periods of drug abstinence, and it can remain high for extended periods(35). Analogous “incubation” phenomena are reported in cocaine-exposed rodents, in which drug-seeking behaviors persist, and are augmented, following cocaine self-administration(3,4). Here we focused on the drug-induced development of so-called “reward-seeking” habits. We found that adolescent mice are more vulnerable to developing cocaine-induced habit-like behavior than adults. These vulnerabilities represent failures in response-outcome conditioning, resulting in a deferral to familiar behavioral patterns, and they incubate. Further, dendritic spines on excitatory deep-layer oPFC pyramidal neurons are lost. Habits are *inducible* by inhibiting Abl-family kinase signaling in the oPFC and *obstructed* using strategies that augment Abl2 signaling.

Neuroplasticity in multiple brain regions, including the oPFC, is implicated in the incubation of drug-seeking behaviors(36). Further, a history of psychostimulant exposure eliminates dendrites and dendritic spines on excitatory neurons in this region(20,37-41). Here we replicate prior findings that subchronic cocaine exposure during adolescence reduces oPFC spine counts in adulthood(20). We further characterize the morphology of remaining spines, revealing that they are, as a population, shorter, and head diameters are smaller, suggesting an overexpression of stubby-type spines, as opposed to motile thin-type, or mature mushroom-type, spines.

In a previous study using adolescent mice, cocaine was administered during adolescence, then a cocaine “challenge” was given prior to euthanasia in adulthood(20). This challenge was absent here, and spine densities were nonetheless reduced, indicating that spine loss can be attributed to early-life drug exposure alone. Remaining spine heads were overall larger in the prior report utilizing a “challenge” injection, but smaller here; thus, previously-reported shifts in head size may be a metaplastic response to cocaine following adolescent exposure. The same population of dendritic spines in mice deficient in the cytoskeletal regulatory protein Abl2 fail to react to cocaine, and these mice rapidly develop cocaine-induced locomotor sensitization and reversal learning deficits(20,42). Together, these findings suggest that cocaine-induced metaplastic spine head enlargement may be protective, strengthening existing oPFC synapses. Indeed, in female mice, a larger population of large-

head spines is associated with resilience to cocaine-induced habits(43), parsimonious with a general model in which cocaine-induced degeneration in oPFC plasticity contributes to failures in impulse control in addiction(6,7,8,11).

Stimulation of cytoskeletal regulatory factors such as Abl2 kinase can grow or stabilize neural structure by blocking RhoA GTPase signaling, a contractile force on the actin cytoskeleton(28). Accordingly, STI-571, an anti-cancer drug that inhibits Abl-family kinases (c-Abl and Abl2), increases actomyosin contractility and reduces actin polymerization-based protrusion(28,34). oPFC-targeted STI-571 infusion here mimicked the behavioral effects of cocaine, suggesting that destabilizing oPFC dendritic structures could be a mechanism by which cocaine biases decision-making strategies towards stimulus-elicited habits. Notably, cocaine- and STI-571-exposed mice also routinely responded less overall. Habit-based behaviors are stimulus- rather than goal-driven; thus, absence of the anticipated reinforcer during the probe test likely energizes responding in typical mice (an “extinction burst”), but not mice insensitive to response-outcome contingency. Also, depressed response rates are often generally observed with oPFC damage(44).

Blockade of cocaine-induced habits

Abl2 influence can be amplified by pharmacologically inhibiting the RhoA substrate Rho-kinase(28), or by silencing NR2B-containing NMDARs, anchoring the Abl2 substrate cortactin in dendritic spines(29). We discovered that both Rho-kinase and NMDAR NR2B inhibition, via fasudil and ifenprodil respectively, rescued action selection strategies following cocaine, strengthening new learning regarding the predictive relationship between actions and their outcomes. The more selective NR2B antagonist CP-101,606 recapitulated these effects, suggesting that ifenprodil acts by inhibiting NR2B-containing NMDARs, rather than via off-target influences, *e.g.*, the α1-adrenergic receptor(45). CP-101,606 may have hallucinogenic properties, however(46), potentially making it a poor candidate for use as a therapeutic adjunct in treating drug use disorders.

Ifenprodil regulates other cocaine-mediated behaviors, reducing cocaine-induced psychomotor sensitization and convulsion in adolescent and adult rodents(47,20). It also decreases neural excitability in the ventral tegmental area after cocaine(48). oPFC-targeted infusions of STI-571 blocked the effects of systemic ifenprodil treatment here, indicating that the inhibition of cocaine-induced behavioral inflexibility by ifenprodil requires intact Abl-family signaling in the oPFC. Thus, ifenprodil appears to impact multiple cortico-meso-limbic regions implicated in addiction, mitigating behavioral vulnerabilities to cocaine.

While adolescent cocaine exposure potently regulated outcome-based decision making, subchronic exposure to amphetamine had no effects. In another report, amphetamine exposure from P21-35 increased dendritic spine density on deep-layer medial prefrontal cortical (mPFC) neurons(49), but only transiently, unlike with cocaine here. We used a higher dose; nonetheless, we identified no durable behavioral consequences, reminiscent of this (lack of) spine change(49). Another possible factor is that cocaine inhibits the reuptake of serotonin, while the effects of amphetamine are weak(50). The suppressive effects of cocaine on adolescent play behavior have been attributed, in part, to actions on serotonin systems(51). Thus, cocaine-induced modifications to serotonin signaling may also contribute

to behavioral inflexibility following adolescent cocaine exposure. Finally, it is also possible that other doses of amphetamine could induce habit biases.

Ifenprodil reduces cue-induced cocaine seeking

We extended our investigation to the cue-induced reinstatement of cocaine seeking, an animal model of relapse. We used young-adult, rather than adolescent, mice self-administering cocaine, motivated by evidence that cocaine self-administration in adulthood decreases dendritic spine density in the mature oPFC(41), recapitulating the sustained effects of subchronic cocaine exposure during adolescence [fig.1,2,(20)]. Additionally, placing intravenous catheters in adolescent mice poses considerable technical challenges. As in the reinstatement of alcohol-, nicotine-, and heroin-seeking behaviors(31,32,33), ifenprodil reduced cue-induced cocaine seeking. A key methodological difference, though, is that we paired ifenprodil with extinction training, rather than the reinstatement test, in order to model intervention strategies in which a pharmacological compound might be paired with behavioral therapy. Mice were tested in the reinstatement test drug-free, and a history of ifenprodil buffered against the reinstatement of cocaine seeking.

Interestingly, ifenprodil did not impact response extinction, even though it reduced reinstatement behavior. This phenomenon is not unprecedented: Extinction training following cocaine self-administration inhibits long-term depression (LTD) in the nucleus accumbens core(52). This plasticity reduces cue-induced reinstatement, but does not impact extinction conditioning itself – as with ifenprodil treatment here. Notably, however, ifenprodil impairs the extinction of conditioned fear(53), and infusions into the infralimbic cortex interfere with the extinction of cocaine-conditioned place preference (CPP)(54). Ifenprodil and other NR2B-selective antagonists preferentially inactivate NMDARs containing two N2RB subunits, largely sparing heterotrimers containing one NR2A and one NR2B subunit(55). Accordingly, ifenprodil is primarily active at extrasynaptic sites in the cerebral cortex, a primary source of LTD, such that NR2B-selective NMDAR antagonists can *decrease* LTD(56,57,58). Ifenprodil could therefore have different influences in different brain regions, and also on different forms of conditioning (*e.g.*, stimulus-outcome as in CPP, *vs*. response-outcome here).

Combatting cocaine-induced behavioral vulnerabilities

Throughout the majority of these experiments, infusions and injections were delivered immediately following response-outcome contingency degradation, during a period when mice should be forming new memories — that a familiar behavior is no longer reinforced with a desired outcome. In both rats and non-human primates, temporary inactivation of the basolateral amygdala (BLA) *during* reinforcer devaluation – another commonly-used assay of goal-directed *(vs*. habit-based) decision making – interferes with subsequent decision making(59,60). Additionally, BLA inactivation attenuates the coding properties of oPFC neurons(61), and BLA projections terminate in deep-layer oPFC(62), where spines were lost following cocaine [fig. 1,2,(20)]. Thus, the consolidation or retention of certain forms of outcome-based learning and memory could require BLA→oPFC plasticity, enabling the subsequent expression of goal-directed response selection.

In sum, cocaine-induced dendritic spine loss in the oPFC could weaken sensitivity to BLA inputs, weakening new learning regarding behaviors and their consequences. Simultaneously, plasticity within a BLA→mPFC circuit may strengthen due to cocaine-induced dendritic spine proliferation in the mPFC [reviewed(8), but see(41)]. Increased plasticity here would be expected to facilitate the reinstatement of cocaine-reinforced responding following extinction(63). These biases would together retard the development of new behavioral patterns in cocaine-abusing individuals seeking to develop and maintain a drug-abstinent lifestyle. Pharmacological inhibition of Rho-kinase (by fasudil) could correct these deficiencies by structurally stabilizing oPFC synapses and possibly also dendrites(64), simplified following developmental cocaine(40). Ifenprodil may do the same by mitigating cocaine-induced reductions in dendritic spine density and enabling new learning, as here. Ifenprodil could also normalize NR2B-mediated signaling in the mPFC, where cocaine increases NR2B levels(65). A better understanding of the circuit-level consequences of ifenprodil could lead to novel treatment approaches to drug abuse. Further insights could also be gained from understanding apparent behavioral resiliencies in amphetamine-exposed mice here and those exposed to cocaine from P24-28, a preadolescent period when dendritic spines in the oPFC typically proliferate(20). For example, cocaine may induce a molecular milieu that favors dendritic spine elimination rather than stabilization, and these influences could be mitigated during developmental periods when dendritic spines are innately proliferating (preceding adolescence), but exaggerated when they are pruning (during adolescence).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Courtni Andrews and Amanda Allen for valuable assistance and Drs. Stephen Traynelis, David Weinshenker, and Leonard Howell for valuable feedback. This work was supported by Children's Healthcare of Atlanta, the Emory Egleston Children's Research Center, DA015040, and DA034808. The Yerkes National Primate Research Center is supported by the Office of Research Infrastructure Programs/OD P51OD011132.

References

1. O'Brien MS, Anthony JC. Risk of becoming cocaine dependent: epidemiological estimates for the United States, 2000-2001. Neuropsychopharmacology. 2005; 30:1006–1018. [PubMed: 15785780]
2. Kessler R, Aguilar-Gaxiola S, Berglund P, Caraveo-Anduaga J, DeWit D, Greenfield S, et al. Patterns and predictors of treatment seeking after onset of a substance use disorder. Arch Gen Psychiatry. 2001; 58:1065–1071. [PubMed: 11695954]
3. Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation: Incubation of cocaine craving after withdrawal. Nature. 2011; 412:141–142.
4. Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. Trends Neurosci. 2011; 34:411–420. [PubMed: 21764143]
5. Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci. 2005; 8:1481–1489. [PubMed: 16251991]
6. Torregrossa MM, Corlett PR, Taylor JR. Aberrant learning and memory in addiction. Neurobiol Learn Mem. 2011; 96:609–623. [PubMed: 21376820]
7. Lucantonio F, Stalnaker TA, Shaham Y, Niv Y, Schoenbaum G. The impact of orbitofrontal dysfunction on cocaine addiction. Nat Neurosci. 2012; 15:358–366. [PubMed: 22267164]

8. DePoy LM, Gourley SL. Synaptic cytoskeletal plasticity in the prefrontal cortex following psychostimulant exposure. Traffic. 2015; 16:919–940. [PubMed: 25951902]
9. Calu DJ, Stalnaker TA, Franz TM, Singh T, Shaham Y, Schoenbaum G. Withdrawal from cocaine self-administration produces long-lasting deficits in orbitofrontal-dependent learning in rats. Learn Mem. 2007; 14:325–328. [PubMed: 17522022]
10. Balleine BW, O'Doherty JP. Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology. 2010; 35:48–69. [PubMed: 19776734]
11. Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F. Addiction: beyond dopamine reward circuitry. Proc Natl Acad Sci U S A. 2011; 108:15037–15042. [PubMed: 21402948]
12. Gremel CM, Costa RM. Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. Nat Commun. 2013; 4:2264. [PubMed: 23921250]
13. Gourley SL, Olevska A, Zimmermann KS, Ressler KJ, Dileone RJ, Taylor JR. The orbitofrontal cortex regulates outcome-based decision-making via the lateral striatum. Eur J Neurosci. 2013; 38:2382–2388. [PubMed: 23651226]
14. CASA (The National Center on Addiction and Substance Abuse). CASA analysis of the National Survey on Drug Use and Health (NSDUH), 2009. Rockville, MD: U.S Department of Health and Human Services, Substance abuse and mental health services administration; 2011.
15. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, et al. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron. 2000; 28:41–51. [PubMed: 11086982]
16. Gourley SL, Swanson AM, Jacobs AM, Howell JL, Mo M, DiLeone RJ, et al. Action control is mediated by prefrontal BDNF and glucocorticoid receptor binding. Proc Natl Acad Sci U S A. 2012; 109:20714–20719. [PubMed: 23185000]
17. Swanson AM, Shapiro LP, Whyte AJ, Gourley SL. Glucocorticoid receptor regulation of action selection and prefrontal cortical dendritic spines. Comm Int Biol. 2013; 6:e26068.
18. Barker JM, Torregrossa MM, Taylor JR. Bidirectional modulation of infralimbic dopamine D1 and D2 receptor activity regulates flexible reward seeking. Front Neurosci. 2013; 7:126. [PubMed: 23882177]
19. Butkovich LM, DePoy LM, Allen AG, Shapiro LP, Swanson AM, Gourley SL. Adolescent-onset $GABA_{A\alpha 1}$ silencing regulates reward-related decision making. Eur J Neurosci. 2015; 42:2114–2121. [PubMed: 26096050]
20. Gourley SL, Olevska A, Warren MS, Taylor JR, Koleske AJ. Arg kinase regulates prefrontal dendritic spine refinement and cocaine-induced plasticity. J Neurosci. 2012; 32:2315–2323.
21. Smith JW, Gastambide F, Gilmour G, Dix S, Foss J, Lloyd K, et al. A comparison of the effects of ketamine and phencyclidine with other antagonists of the NMDA receptor in rodent assays of attention and working memory. Psychopharmacology. 2011; 217:255–269. [PubMed: 21484239]
22. Higgins GA, Ballard TM, Enderlin M, Haman M, Kemp JA. Evidence for improved performance in cognitive tasks following selective NR2B NMDA receptor antagonist pretreatment in the rat. Psychopharmacology. 2005; 179:85–98. [PubMed: 15759152]
23. Rosahl TW, Wingrove PB, Hunt V, Fradley RL, Lawrence JM, Heavens RP, et al. A genetically modified mouse model probing the selective action of ifenprodil at the N-methyl-D-aspartate type 2B receptor. Mol Cell Neurosci. 2006; 33:47–56. [PubMed: 16870468]
24. Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev. 2000; 24:417–463. [PubMed: 10817843]
25. Green MR, McCormick CM. Effects of stressors in adolescence on learning and memory in rodent models. Hormones Behav. 2013; 64:364–379.
26. Anthony JC, Petronis KR. Early-onset drug use and risk of later drug problems. Drug Alcohol Depend. 1995; 40:9–15. [PubMed: 8746919]
27. Harris KM, Jensen FE, Tsao B. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: Implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci. 1992; 12:2685–2705. [PubMed: 1613552]
28. Koleske AJ. Molecular mechanisms of dendrite stability. Nat Rev Neurosci. 2013; 14:536–550. [PubMed: 23839597]

29. Lin YC, Yeckel MH, Koleske AJ. Abl2/Arg controls dendritic spine and dendrite arbor stability via distinct cytoskeletal control pathways. J Neurosci. 2013; 33:1846–1857. [PubMed: 23365224]
30. Satoh S, Utsunomiya T, Tsurui K, Kobayashi T, Ikegaki I, Sasaki Y, et al. Pharmacological profile of hydroxyl fasudil as a selective rho kinase inhibitor on ischemic brain damage. Life Sci. 2001; 69:1441–1453. [PubMed: 11531167]
31. Vengeliene V, Bachteler D, Danysz W, Spanagel R. The role of the NMDA receptor in alcohol relapse: A pharmacological mapping study using the alcohol deprivation effect. Neuropharmacology. 2005; 4:822–829.
32. Shen H, Moussawi K, Zhou W, Toda S, Kalivas PW. Heroin relapse requires long-term potentiation-like plasticity mediated by NMDA2b-containing receptors. Proc Natl Acad Sci U S A. 2011; 108:19407–19412. [PubMed: 22084102]
33. Gipson CD, Reissner KJ, Kupchik YM, Smith AC, Stankeviciute N, Hensley-Simon ME, et al. Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. Proc Natl Acad Sci U S A. 2013; 110:9124–9129. [PubMed: 23671067]
34. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-Kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther. 2000; 295:139–145. [PubMed: 10991971]
35. Gawin FH, Kleber HD. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. Arch Gen Psychiatry. 1986; 43:107–113. [PubMed: 3947206]
36. Fanous S, Goldart EM, Theberge FR, Bossert JM, Shaham Y, Hope BT. Role of orbitofrontal cortex neuronal ensembles in the expression of incubation of heroin craving. J Neurosci. 2012; 32:11600–11609. [PubMed: 22915104]
37. Crombag HS, Gorny G, Li Y, Kolb B, Robinson TE. Opposite effects of amphetamine self-administration experience on dendritic spines in the medial and orbital prefrontal cortex. Cereb Cortex. 2005; 15:341–348. [PubMed: 15269111]
38. Muhammad A, Kolb B. Maternal separation altered behavior and neuronal spine density without influencing amphetamine sensitization. Behav Brain Res. 2011; 223:7–16. [PubMed: 21515311]
39. Muhammad A, Kolb B. Mild prenatal stress-modulated behavior and neuronal spine density without affecting amphetamine sensitization. Dev Neurosci. 2011; 33:85–98. [PubMed: 21576912]
40. DePoy LM, Perszyk RE, Zimmermann KS, Koleske AJ, Gourley SL. Adolescent cocaine exposure simplifies orbitofrontal cortical dendritic arbors. Front Pharmacol. 2014; 5:228. [PubMed: 25452728]
41. Radley JJ, Anderson RM, Cosme CV, Glanz RM, Miller MC, Romig-Martin SA, et al. The contingency of cocaine administration accounts for structural and functional medial prefrontal deficits and increased adrenocortical activation. J Neurosci. 2015; 35:11897–11910. [PubMed: 26311772]
42. Gourley SL, Koleske AJ, Taylor JR. Loss of dendrite stabilization by the Abl-related gene (Arg) kinase regulates behavioral flexibility and sensitivity to cocaine. Proc Natl Acad Sci U S A. 2009; 106:16859–16864. [PubMed: 19805386]
43. DePoy LM, Allen AG, Gourley SL. Adolescent cocaine self-administration induces habit behavior in adulthood: Sex differences and structural consequences. Trans Psych. 2016; 6:e875.
44. Stalnaker TA, Cooch NK, Schoenbaum G. What the orbitofrontal cortex does not do. Nat Neurosci. 2015; 18:620–627. [PubMed: 25919962]
45. Chenard BL, Shalaby IA, Koe BK, Ronau RT, Butler TW, Prochniak MA, et al. Separation of alpha 1 adrenergic and N-methyl-D-aspartate antagonist activity in a series of ifenprodil compounds. J Med Chem. 1992; 34:3085–3090.
46. Nicholson KL, Mansbach RS, Menniti FS, Balster RL. The phencyclidine-like discriminative stimulus effects and reinforcing properties of the NR2B-selective N-methyl-D-aspartate antagonist CP-101 606 in rats and rhesus monkeys. Behav Pharmacol. 2007; 18:731–743. [PubMed: 17989511]
47. Witkin JM, Acri JB. Effects of ifenprodil on stimulatory, discriminative stimulus, and convulsant effects of cocaine. Behav Pharmacol. 1995; 6:245–253. [PubMed: 11224333]

48. Yuan T, Mameli M, O'Connor EC, Dey PN, Verpelli C, Sala C, et al. Expression of cocaine-evoked synaptic plasticity by GluN3A-containing NMDA receptors. Neuron. 2013; 80:1025–1038. [PubMed: 24183704]
49. Tendilla-Beltrán H, Arroyo-García LE, Diaz A, Camacho-Abrego I, de la Cruz F, Rodríguez-Moreno A, et al. The effects of amphetamine exposure on juvenile rates on the neuronal morphology of the limbic system at prepubertal, pubertal and postpubertal ages. J Chem Neuroanat. 2016; 77:68–77. [PubMed: 27208629]
50. Ritz MC, Kuhar MJ. Relationship between self-administration of amphetamine and monoamine receptors in brain: comparison with cocaine. J Pharmacol Exp Ther. 1989; 248:1010–1017. [PubMed: 2703961]
51. Achterberg EJM, Trezza V, Siviy SM, Schrama L, Schoffelmeer ANM, Vanderschuren LJMJ. Amphetamine and cocaine suppress social play behavior in rats through distinct mechanisms. Psychopharmacology. 2014; 231:1503–1515. [PubMed: 24057815]
52. Knackstedt LA, Moussawi K, Lalumiere R, Schwendt M, Klugmann M, Kalivas PW. Extinction training after cocaine self-administration induced glutamatergic plasticity to inhibit cocaine seeking. J Neurosci. 2010; 30:7984–7992. [PubMed: 20534846]
53. Sotres-Bayon F, Bush DE, LeDoux JE. Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. Neuropsychopharmacology. 2007; 32:1929–1940. [PubMed: 17213844]
54. Otis JM, Fitzgerald MK, Mueller D. Infralimbic BDNF/TrkB enhancement of GluN2B currents facilitates extinction of a cocaine-conditioned place preference. J Neurosci. 2014; 34:6057–6064. [PubMed: 24760865]
55. Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci. 2013; 14:383–400. [PubMed: 23686171]
56. Stocca G, Vicini S. Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. J Physio. 1998; 507:13–24.
57. Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, et al. Differential roles of NR2A and NR2B-contiaining NMDA receptors in cortical long-term potentiation and long-term depression. J Neurosci. 2004; 24:7821–7828. [PubMed: 15356193]
58. Tovar KR, Westbrook GL. The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. J Neurosci. 1999; 19:4180–4188. [PubMed: 10234045]
59. Wellman LL, Gale K, Malkova L. GABAA-mediated inhibition of basolateral amygdala blocks reward devaluation in macaques. J Neurosci. 2005; 25:4577–4586. [PubMed: 15872105]
60. West EA, Forcelli PA, Murnen AT, McCue DL, Gale K, Malkova L. Transient inactivation of basolateral amygdala during selective satiation disrupts reinforcer devaluation in rats. Behav Neurosci. 2012; 125:563–574.
61. Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends on input from basolateral amygdala. Neuron. 2003; 39:855–867. [PubMed: 12948451]
62. Ghashghei HT, Barbas H. Pathways for emotion: Interactions of prefrontal and anterior temporal pathways in the amygdala of the rhesus monkey. Neuroscience. 2002; 115:1261–1279. [PubMed: 12453496]
63. Stefanik MT, Kalivas PW. Optogenetic dissection of basolateral amygdala projections during cue-induced reinstatement of cocaine seeking. Front Behav Neurosci. 2013; 7:213. [PubMed: 24399945]
64. Couch BA, DeMarco GJ, Gourley SL, Koleske AJ. Increased dendrite branching in AβPP/PS1 mice and elongation of dendrite arbors by fasudil administration. J Alzheimers Dis. 2010; 20:1003–1008. [PubMed: 20413901]
65. Ben-Shahar O, Obara I, Ary AW, MA N, Mangiardi MA, Medina RL, et al. Extended daily access to cocaine results in distinct alterations in homer 1b/c and NMDA receptor subunit expression within the medial prefrontal cortex. Synapse. 2009; 63:598–609. [PubMed: 19306440]
66. LaLumiere RT, Niehoff KE, Kalivas PW. The infralimibic cortex regulates the consolidation of extinction after cocaine self-administration. Learn Mem. 2010; 17:168–175. [PubMed: 20332188]

67. Rosen GD, Williams AG, Capra JA, Connolly MT, Cruz B, Lu L, et al. The mouse brain library. International Mouse Genome Conference. 2000; 14:166. www.Mbl.Org.

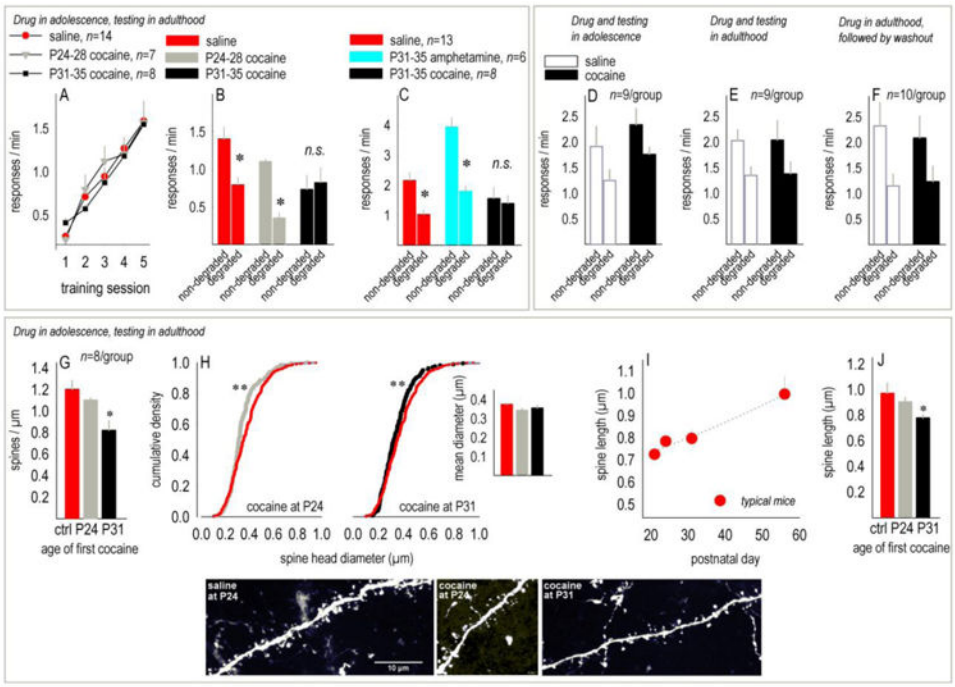

Figure 1. Mice exposed to cocaine as adolescents are insensitive to changes in response-outcome contingencies as adults, and oPFC dendritic spines are modified

(a) We exposed mice to cocaine from P24-28 (preadolescence) or P31-35 (early adolescence). We then trained mice to generate 2 nose poke responses for food reinforcers as adults. Response acquisition curves represent both responses/min, and there were no side biases throughout. (b) Mice with a history of early-adolescent cocaine exposure were later unable to differentiate between responses that were likely, *vs.* unlikely, to be reinforced ("non-degraded" *vs.* "degraded"). Instead, these mice engaged two familiar responses equally. (c) Amphetamine administered from P31-35 did not induce this behavioral inflexibility. (d) Separate adolescent mice were exposed to cocaine, then tested *without* a drug washout period. A main effect of the response choice indicated that these mice could differentiate between the responses that were more, *vs.* less, likely to be reinforced in a goal-directed manner. (e) Subchronic cocaine exposure in adult mice also did not impact instrumental response selection, including (f) when mice were given a drug washout period (as in adolescent mice). (g) Dendritic spines on pyramidal neurons in deep-layer oPFC were imaged in adult mice previously exposed to cocaine from P24-28 or P31-35. Density was reduced following P31-35 exposure. (h) Cocaine shifted dendritic spine head diameter cumulative density curves leftward, indicating that dendritic spine heads were also generally smaller in adult mice with a history of cocaine exposure, regardless of age of exposure. Average diameters are inset. (i) Average dendritic spine length increases in the oPFC during adolescent development, however (j) dendritic spines in mature mice with a history of adolescent (P31-35) cocaine exposure were shorter. Representative dendrites are below. Scale bar=10 µm. Means+SEMs,*p<0.05,**p<0.001. Group sizes are indicated.

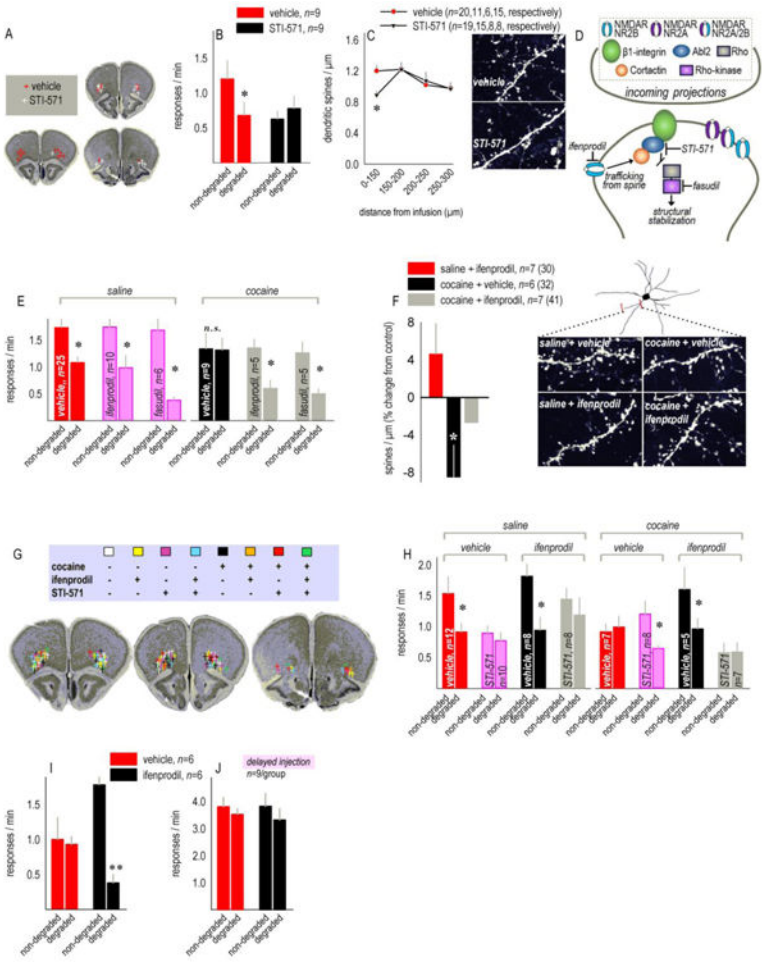

Figure 2. Bidirectional regulation of goal-directed action

(a) We trained mice to respond for food reinforcers (fig.S1), then infused into the oPFC STI-571 following instrumental contingency degradation. Infusion sites are represented on images from the Mouse Brain Library (67). (b) STI-571 caused an inability to select between actions that were likely, *vs.* unlikely, to be reinforced ("non-degraded" *vs.* "degraded"). (c) Dendritic spine densities were reduced within 150 µm of the infusion sites. Representative dendrites are adjacent. (d) Abl2 activity is enriched by inhibiting NR2B-containing NMDARs, blocking cortactin from being trafficked from the spine. The inhibition of Rho-kinase also amplifies Abl2 influence (see 28,29). These events can be induced pharmacologically by ifenprodil and fasudil, respectively, ultimately stabilizing neural structure. (e) Both ifenprodil and fasudil blocked cocaine-induced behavioral inflexibility, indicated by a preference for the response that was more, *vs.* less, likely to be reinforced. (f) We focused the rest of our report on ifenprodil, revealing that ifenprodil mitigated cocaine-induced reductions in dendritic spine density in the oPFC (0 represents no change from control). Inset: An oPFC neuron with distal branches highlighted and representative spines. (g-h) Further, STI-571 infusions occluded the effects of ifenprodil in enriching goal-directed action selection following adolescent cocaine exposure. Infusion sites are represented on images from the Mouse Brain Library (67). (i) Separate, drug-naïve mice were trained extensively to nose poke for food reinforcers (fig.S1). Subsequently,

control mice developed habit-based response strategies, as expected. Ifenprodil restored goal-directed response selection. (j) When ifenprodil treatment was delayed 4 hours following instrumental contingency degradation, it had no effect. Means+SEMs, *p<0.05,**p<0.001. Group sizes are indicated; numbers within parentheses refer to the number of dendrites sampled.

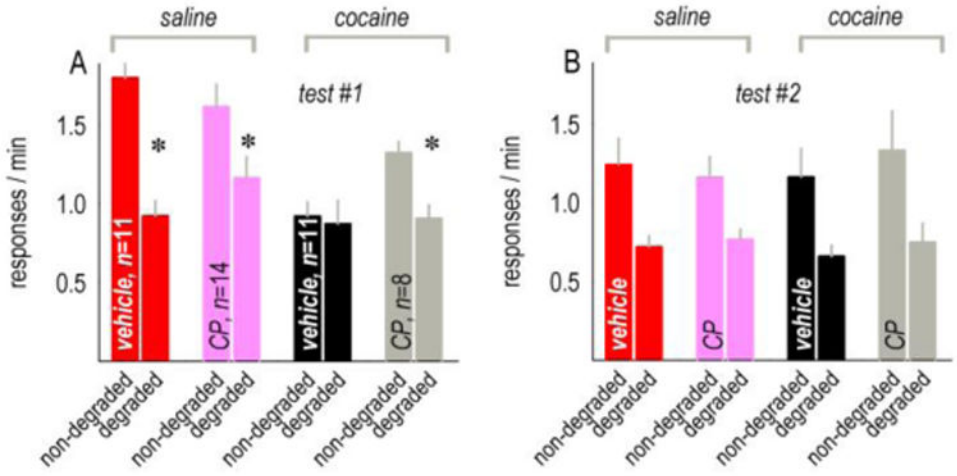

Figure 3. The NMDAR NR2B-selective antagonist CP-101,606 occludes cocaine-induced behavioral inflexibility

(a) Mice were trained to respond for food reinforcers, with no differences between groups (fig.S1). Mice with a history of cocaine exposure subsequently failed to differentiate between responses that were likely, *vs.* unlikely, to be reinforced, as expected. CP-101,606 ("CP") blocked these deficiencies. (b) With additional testing, cocaine-exposed mice were ultimately able to differentiate between responses, indicating that cocaine delays, but does not fully block, the development and expression of goal-directed response strategies. Means +SEMs, *$p<0.05$ following interactions. Group sizes are indicated.

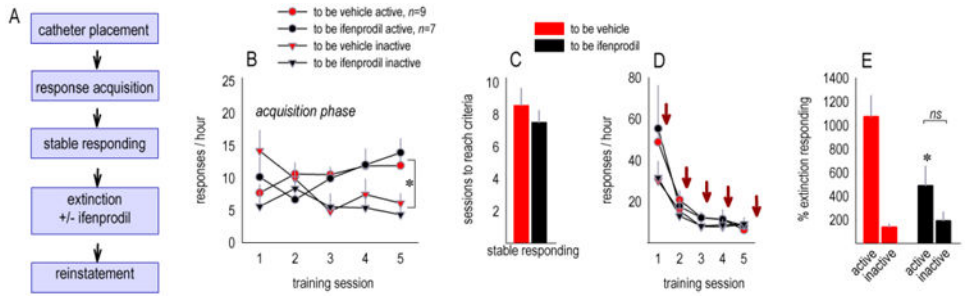

Figure 4. Ifenprodil reduces the cue-induced reinstatement of cocaine seeking

(a) Experimental timeline. (b) Cocaine-reinforced response rates during the response acquisition phase did not differ between mice that would ultimately be treated with vehicle *vs.* ifenprodil. (c) Mice required the same amount of training to develop stable response rates, regardless of whether they would or would not be ultimately treated with ifenprodil. (d) Further, ifenprodil treatment immediately following each extinction training session (arrows) had no effects on response extinction. (e) Nonetheless, ifenprodil reduced cocaine-seeking behaviors when mice were primed with cocaine-associated cues in a reinstatement test. Ifenprodil-treated mice responded less on the previously active aperture than vehicle-treated mice, and additionally, their responding on the active aperture did not significantly differ from responding on the inactive apertures ($p=0.086$ between apertures in ifenprodil mice; $p<0.001$ for the same comparison in vehicle-treated mice). Ifenprodil was not on-board at this time. Means+SEMs, *$p<0.05$. Group sizes are indicated.

Table 1

Summary of experiments

The timing of experimental events is indicated. “P” refers to postnatal day. Injections were systemic unless otherwise noted. “Training” refers to the conditioning session in which a familiar response-outcome contingency was violated, except in the final main text experiment, in which we are referring to extinction training sessions.

Group	Figure	Age of cocaine exposure	Age of behavioral testing	Injections/infusions other than cocaine	Timing of injections	Age of euthanasia & post-mortem procedure
1	1a,b	P24-28	P56			
2	1a,b	P31-35	P56			
3	1c	P31-35, COC or amphetamine	P56			
4	1d	P31-35	P36			
5	1e	P56-60	P67			
6	1f	P56-60	P81			
8	1g,h,j	P24-28	n/a			P56 → image dendritic spines
9	1g,h,j	P31-35	n/a			P56 → image dendritic spines
10	1i	n/a	n/a			P21, 24, 31, and 56 → image dendritic spines
11	2a-c	n/a	P56	intracranial vehicle or STI-571	immediately post-training	~P70 → histology; image dendritic spines
12	2e	P31-35	P56	vehicle, ifenprodil or fasudil	immediately post-training	
13	2f	P31-35	P56	vehicle or ifenprodil	immediately post-training	~P64 → image dendritic spines
14	2g,h	P31-35	P56	vehicle or ifenprodil/intracranial vehicle or STI-571	immediately post-training	~P70 → histology
15	2i	n/a	P56	vehicle or ifenprodil	immediately post-training	
16	2j	n/a	P56	vehicle or ifenprodil	4 hours post-training; timing adapted from (66)	
17	3	P31-35	P56	vehicle or CP-101,606	immediately post-training	
18	4	P63 start	P56	vehicle or ifenprodil	Immediately post-extinction training	
19	S3	n/a	~P70	vehicle or fasudil	immediately before test	