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Over-inhibition of Corticostriatal Activity following Prenatal Cocaine Exposure

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Abstract

Objective—Prenatal cocaine exposure (PCE) can cause persistent neuropsychological and motor abnormalities in affected children, but the physiological consequences of PCE remain unclear. Conclusions drawn from clinical studies can sometimes be confounded by poly-substance abuse and nutritional deprivation. However, existing observations suggest that cocaine exposure *in utero*, as in adults, increases synaptic dopamine and promotes enduring dopamine-dependent plasticity at striatal synapses, altering behaviors and basal ganglia function.

Methods—We used a combination of behavioral measures, electrophysiology, optical imaging, and biochemical and electrochemical recordings to examine corticostriatal activity in adolescent mice exposed to cocaine *in utero*.

Results—We show that PCE caused abnormal dopamine-dependent behaviors, including heightened excitation following stress and blunted locomotor augmentation to repeated treatment with amphetamine. These abnormal behaviors were consistent with abnormal GABA interneuron function, which promoted a reversible depression in corticostriatal activity. PCE hyperpolarized and reduced tonic GABA currents in both fast-spiking and PLTS-type GABA interneurons to increase tonic inhibition at GABA_B receptors on presynaptic corticostriatal terminals. While D2 receptors paradoxically increased glutamate release following PCE, normal corticostriatal modulation by dopamine was reestablished with a GABA_AR antagonist.

Interpretation—The dynamic alterations at corticostriatal synapses that occur in response to PCE parallel the reported effects of repeated psychostimulants in mature animals, but differ in being specifically generated through GABA. Our results indicate that approaches which normalize GABA and D2 receptor-dependent synaptic plasticity may be useful for treating the behavioral effects of PCE and other developmental disorders that are generated through abnormal GABAergic signaling.

Introduction

PCE can cause debilitating neuropsychological and motor abnormalities in humans and is an important public health concern.¹ In spite of prevention-related measures, over 4% of pregnant women in the United States use illicit drugs,² with up to 1% using cocaine.¹ Cocaine readily crosses the placenta³ and can cause withdrawal symptoms in neonates and cognitive and behavioral abnormalities in adolescence.^{4,5} Unfortunately, the prospect for treating affected children remains poor, as the drug's effect on the developing nervous system remains unclear.⁶

Observations in the clinic^{1,4,5} and laboratory^{7–10} suggest that abnormal symptoms and signs following PCE reflect alterations in corticostriatal function. The striatum is the primary nucleus for cortical information entering the basal ganglia and regulates motor control, cognition, and habit learning.^{11,12} Attention to salient behavioral cues, as well as the selection and execution of movements and decisions, requires the striatum to collect cortical signals, modulate information, and activate appropriate corticofugal pathways.¹³ Signal integration is thought to occur at a 'striatal microcircuit', where glutamate from cortical inputs, dopamine from nigrostriatal terminals, and gamma-aminobutyric acid (GABA) from striatal interneurons interact at the dendritic spines of striatal medium spiny projection neurons (MSNs) to select behaviorally-relevant synapses (Fig 1A).^{12,14–16} Here we show that PCE causes abnormal dopamine-dependent motor behaviors and striatal synaptic plasticity in adolescent mice through abnormal GABA interneuron function, providing insights into novel therapeutic approaches that may improve outcome in children with PCE.

Materials and Methods

Animals

Procedures were approved by the University of Washington Institutional Animal Care and Use Committee and are detailed in the Supplementary Methods. Timed-pregnant Swiss Webster-strain (n=78), C57B16-strain (n=9), and Lhx6-GFP BAC transgenic (n=10; MMRRRC-GENSAT project, www.gensat.org) dams received either cocaine (20mg/kg, s.q.) or saline twice daily from embryonic day 8 through 18.⁷ As cocaine causes anorexia,⁷ controls included a saline-treated, pair-fed group (SPF) whose nutritional intake was matched to that of cocaine-treated dams, and a saline-treated group that were allowed to feed ad libitum (saline). Litters were fostered to an untreated surrogate dam at birth (P0), weaned by P22, and utilized as indicated below.

Behavior

Tail-flick, rotarod, open-field, locomotor, and tail suspension tests were performed in 181 mice, aged 60–80 days, as described^{17–21} in the Supplementary Methods.

Electrophysiology

Whole-cell electrophysiological recordings were made in 318 neurons from 125 mice aged 30–32 and 55–94 days, as described²² in the Supplementary Methods.

Multiphoton Optical Imaging with FM1-43

Optical recordings of presynaptic corticostriatal release were made in slices from 46 mice, aged 30–33 days and 60–80 days, as described¹⁶ in the Supplementary Methods.

Western Blotting

Tissue was extracted from the dorsal striatum of 14 mice, aged 63–70 days and biotinylated for both total and surface receptor (R) expression of GABA_BR subunit proteins, as described²³ in the Supplementary Methods.

Fast Scan Cyclic Voltammetry

Dopamine efflux was measured at sub-second resolution in striatal slices from 14 mice, aged 63–79 days using cyclic voltammetry, as described^{15,20,24} in the Supplementary Methods.

Statistics

Values given in the text and in the figures are Mean ± SEM. Differences, considered significant if $p < 0.05$, were assessed with appropriate *t*-tests, ANOVAs or the non-parametric Mann-Whitney test (see Supplementary Methods).

Results

Abnormal growth and dopamine-dependent behaviors

Timed-pregnant cocaine-exposed ($n=19$) and SPF dams ($n=17$) consumed similar quantities of food throughout gravidity ($p=0.95$), but 14% less than saline dams ($n=20$; $p < 0.001$, *t*-test; Fig 2A). Maternal weights reflected these differences in food intake, as saline dams gained more weight than either cocaine or SPF dams ($p < 0.001$, repeated-measures ANOVA; Fig 2B). Following birth, cocaine-exposed pups ($n=59$) weighed less than either saline ($n=96$) or SPF pups ($n=51$) until week 6 ($p < 0.01$, *t*-test; Fig 2C), while the cranial diameter of saline pups ($n=100$) was greater than that of SPF ($n=24$) or cocaine pups ($n=35$) after week 1 ($p < 0.01$, *t*-test; Fig 2D). The reduction in postnatal weight was dependent on PCE, while the smaller head size was a consequence of nutrition.

Dopamine-dependent reflexes and behaviors were assessed in adolescent mice. Cocaine-exposed mice ($n=19$) showed increased nociceptive latencies on tail-flick testing compared to either saline ($n=27$) or SPF mice ($n=20$; $p < 0.001$, Mann-Whitney; Fig 3A). Rotarod testing for motor coordination revealed increasing falling latencies for saline ($n=26$), SPF ($n=20$), and cocaine-exposed mice ($n=8$; $p < 0.001$, repeated-measures ANOVA; Fig 3B), consistent with motor learning.¹⁹ However, falling latencies in cocaine-exposed mice decreased in later trials ($p < 0.05$ compared to either saline or SPF mice, ANOVA) and cocaine mice gripped the rod more frequently than either saline or SPF mice ($p < 0.001$, Mann-Whitney; Fig 3C).

Locomotion in the open-field was similar in saline ($n=35$), SPF ($n=38$), and cocaine-exposed mice ($n=24$; Fig 3D). Following a saline injection, the ambulations of saline and SPF mice remained similar while cocaine-exposed mice became more active ($p < 0.001$, *t*-test). In the absence of injection, similar responses were found in all groups (not shown).

To test for dopamine-dependent plasticity,²⁰ locomotor responses were measured in response to repeated amphetamine (2mg/kg/d i.p.). Saline ($n=14$), SPF ($n=20$), and cocaine-exposed mice ($n=12$) all demonstrated locomotor sensitization with an increase in locomotor activity following repeated amphetamine ($p < 0.01$, repeated-measures ANOVA). Similar to prior studies,²⁵ cocaine-exposed mice had blunted locomotor augmentation to repeated amphetamine ($p < 0.001$, repeated-measures ANOVA; Fig 3E), but comparable sensitized responses to amphetamine challenges in withdrawal.

As a control experiment to test for hypodopaminergia²¹ and depression,²⁶ mice were suspended by the tail and time spent immobile was measured. While there was no overall

difference between saline (n=49), SPF (n=29), and cocaine mice (n=29; Fig 3F), both SPF and cocaine mice displayed small increases in immobility compared to saline mice, suggesting an effect of nutritional deficiency as opposed to PCE.

Presynaptic inhibition of corticostriatal activity

These behaviors suggested that PCE might alter dopamine-dependent excitatory neurotransmission within the dorsal (motor) striatum.²⁷ Whole-cell electrophysiological recordings in MSNs from SPF and cocaine-exposed mice revealed similar passive and active membrane properties (Fig 4A and Supplementary Table S1). However, compared to SPF mice, neurons from cocaine-exposed mice were 36% less responsive to current injection ($p < 0.001$, ANOVA; Fig 4B) and cortical stimulation > 0.6 mA evoked lower amplitude excitatory postsynaptic currents (eEPSC; $p < 0.05$, two-way ANOVA; Fig 4C and D). This inhibition of corticostriatal activity following PCE was likely of presynaptic origin,²⁸ since the eEPSC paired-pulse ratio (PPR) was higher in cocaine (1.2 ± 0.05 ; n=49 cells) than in SPF (1.07 ± 0.03 ; n=60; $p = 0.01$) and the frequency of miniature (m) EPSCs was 18% less in cocaine-exposed cells (n=19) compared to SPF (n=14; $p = 0.03$, *t*-test), while the cumulative mEPSC amplitude distributions were unchanged (Fig 4E).

Exocytosis from corticostriatal terminals was directly examined by multiphoton microscopy using the endocytic tracer FM1-43.¹⁵ The halftime of FM1-43 release was 14% lower in cocaine-treated mice (n=125 puncta; $p = 0.02$, Mann-Whitney) and compared to saline (n=102) or SPF (n=100), PCE selectively inhibited the subset (~50%) of cortical terminals with a low probability of release, while the faster-releasing terminals remained unperturbed (Fig 4F–H). This synaptic depression was long-lasting, since exocytosis in younger 30-day-old mice was 18% lower in cocaine ($t_{1/2} = 245$ sec; n=114) compared to saline ($t_{1/2} = 201$ sec; n=68; $p = 0.01$, Mann-Whitney not shown).

GABA_BR-dependent over-inhibition at corticostriatal synapses

We tested whether this presynaptic depression might occur through GABAergic neurotransmission, which regulates presynaptic corticostriatal activity¹⁴ via metabotropic GABA_BRs located on corticostriatal terminals.²⁹ Tonic inhibition by GABA_BRs was absent in SPF mice, since the GABA_BR antagonist CGP52432 (10 μ M) did not change the eEPSC amplitude (Fig 4D) or the frequency of mEPSCs ($10 \pm 14\%$; n=7 cells; Fig 5A). However, CGP52432 prevented the tonic inhibition of MSNs from cocaine-exposed mice (Fig 4D) and increased the frequency of mEPSCs ($19 \pm 3\%$; n=6; $p = 0.02$, paired *t*-test; Fig 5B).

To determine if PCE might change the sensitivity of presynaptic GABA_BRs, eEPSCs from MSNs were measured in response to cortical stimulation with 50 ms paired-pulses, applied every 30 sec.³⁰ In MSNs from SPF mice, the GABA_BR agonist baclofen (5 μ M) decreased the amplitude of the first current of the pair ($-67 \pm 3\%$; $p = 0.002$) and the PPR increased ($44 \pm 8\%$; n=14; $p < 0.001$, paired *t*-test; Fig 5C), consistent with strong presynaptic inhibition by GABA_BRs.³⁰ In cocaine-exposed mice, baclofen also decreased the amplitude of the first eEPSC ($-79 \pm 4\%$; n=8; $p = 0.03$) and the PPR increased ($61 \pm 6\%$; $p = 0.01$, paired *t*-test; Fig 5D). Over a range of concentrations, baclofen depressed corticostriatal activity to a greater degree in cocaine-exposed MSNs ($p < 0.05$, 2-way ANOVA; Fig 5E and F), suggesting that PCE increases GABA_BR sensitivity.

Overexpression of GABA_BR1a-receptor subunits

Western blots determined striatal GABA_BR subunit expression in the dorsal striatum and the membrane surface protein pool was detected with biotinylation. Results showed a $25 \pm 5\%$ increase in the GABA_BR1a-receptor subunit surface protein membrane pool from cocaine-exposed striatum (1.41 ± 0.3) compared to SPF (1 ± 0.1 ; n=7 mice each, $p = 0.02$, *t*-test; Fig 6).

The total protein level of GABA_BR1a and levels of both GABA_BR1b and GABA_BR2 were unchanged, indicating that PCE selectively increases the expression of GABA_BR1a-receptor subunits, which are preferentially expressed on corticostriatal terminals.³¹

Paradoxical Excitation by D2Rs is dependent on GABA_BRs

Dopamine modulates corticostriatal activity through D2Rs¹⁶ and in MSNs from SPF mice, the D2R agonist quinpirole decreased the amplitude of the first eEPSC ($-15\pm 7\%$; $n=9$ cells; $p=0.004$) and increased the PPR ($30\pm 8\%$; $p=0.01$, paired t -test; Fig 7A). In cocaine mice, quinpirole paradoxically increased the eEPSC amplitude ($43\pm 19\%$; $n=8$; $p=0.02$) and decreased the PPR ($-25\pm 6\%$; $p=0.05$, paired t -test; Fig 7B). This aberrant excitation of corticostriatal activity by D2Rs following PCE was likely presynaptic since quinpirole reduced the frequency of mEPSCs in SPF cells ($-18\pm 6\%$; $n=7$; $p=0.003$; Fig 7C), but enhanced their frequency in cocaine-exposed neurons ($38\pm 14\%$; $n=8$; $p=0.04$, paired t -test; Fig 7D). Similar plasticity was found in MSNs from mice exposed to half the dose of cocaine and also in younger 30-day-old C57Bl6-strain mice (Supplementary Data and Fig S1).

These changes in corticostriatal activity were accompanied by long-term adaptations in dopamine transmission, as measurements of electrically-evoked dopamine release and reuptake using cyclic voltammetry showed that PCE produced region-specific alterations in phasic dopamine release without affecting clearance (Supplementary Data and Fig S2). However, optical experiments in slices from saline and SPF mice, confirmed that both the dopamine releaser amphetamine¹⁵ ($10\mu\text{M}$) and quinpirole reduced exocytosis by specifically modulating glutamatergic inputs with a low probability of release (Fig 7E, Supplementary Data and Fig S3), but boosted release from those same synapses in slices from cocaine-exposed mice. Consistent with the lack of D1Rs on cortical terminals within the dorsal striatum,¹⁵ D1R ligands did not change exocytosis or the modulatory response to amphetamine (Supplementary Data and Fig S4).

While D2Rs inhibit corticostriatal release in untreated mice,¹⁵ they also suppress GABA interneuron function,³² and might increase corticostriatal activity by reducing tonic inhibition at sensitized GABA_BRs. In MSN from SPF mice, inhibition by D2Rs was not dependent on GABA_BRs, as the GABA_BR antagonist CGP52432 did not change the eEPSC amplitude ($-5\pm 3\%$; $n=6$) or the PPR ($13\pm 5\%$), whereas CGP52432 with quinpirole decreased the eEPSC amplitude ($-23\pm 4\%$; $p<0.03$) and increased the PPR ($33\pm 11\%$; $p<0.02$, compared with vehicle or CGP52432, paired t -test; Fig 7F). In MSNs from cocaine-exposed mice however, CGP52432 was excitatory, as the eEPSC amplitude increased ($38\pm 15\%$; $n=8$; $p=0.03$) and the PPR decreased ($-26\pm 6\%$; $p=0.02$, paired t -test; Fig 7G). CGP52432 with quinpirole remained excitatory, but the eEPSC amplitude decreased ($-18\pm 8\%$; $p<0.04$) and the PPR increased ($19\pm 12\%$; $p<0.05$, compared with vehicle or CGP52432, paired t -test), possibly due to concurrent corticostriatal inhibition by D2Rs.³⁰

GABA_AR blockade prevents D2R-dependent excitation

GABA_ARs promote tonic inhibition of GABA interneurons,³³ but are absent from presynaptic corticostriatal terminals.²⁹ In MSNs from SPF mice, the GABA_AR antagonist bicuculline ($10\mu\text{M}$) did not change the eEPSC amplitude ($5\pm 3\%$; $n=9$) or the PPR ($12\pm 7\%$; Fig 7H) and D2Rs remained inhibitory, as quinpirole in the presence of bicuculline decreased the eEPSC amplitude ($-15\pm 3\%$; $p=0.02$) and increased the PPR ($50\pm 9\%$; $p<0.01$, compared with vehicle or bicuculline, paired t -test). In cocaine cells, bicuculline did not change the eEPSC amplitude ($2\pm 7\%$; $n=9$; $p=0.3$) or the PPR ($3\pm 4\%$; $p=0.6$), but bicuculline blocked excitation by D2Rs since quinpirole with bicuculline reduced the eEPSC amplitude

($-25\pm 8\%$; $p<0.02$) and increased the PPR ($38\pm 7\%$; $p<0.04$, compared with vehicle or bicuculline, paired t -test; Fig 7I).

PCE alters GABA interneuron function

As results indicated that cocaine-induced corticostriatal plasticity is generated through GABA interneurons, we used Lhx6-GFP transgenic mice to target striatal fast-spiking (FS) and persistent-low-threshold-spiking (PLTS) interneurons. Whole-cell voltage- and current-clamp recordings identified FS and PLTS interneurons by their distinctive physiological properties^{14,34} and showed that PCE lowered their resting membrane potentials (FS, -11.6% , $p=0.04$; PLTS, -18% , $p=0.02$) and action potential thresholds (FS, -18% , $p=0.02$; PLTS, -27% , $p=0.001$, compared to saline, t -test; Supplementary Table S2–S4, Fig 8A and B).

In saline-exposed mice, ambient GABA inhibited interneurons as the GABA_AR antagonist bicuculline depolarized both FS ($32\pm 2\%$, $n=4$, $p<0.001$) and PLTS cells ($26\pm 8\%$, $n=5$, $p=0.04$, paired t -test; Fig 8C and D). However, cocaine-exposed FS and PLTS cells demonstrated little depolarization following bicuculline (FS, $2\pm 3\%$, $p=0.4$; PLTS, $4\pm 2\%$, $p=0.1$), consistent with the reported reduction in GABA_AR subunit expression following PCE.^{23,27}

D2Rs can modify tonic GABA inhibition through G-protein-coupled phosphorylation of GABA_AR subunits by PKA.³⁵ The D2R agonist quinpirole did not change the membrane potential in FS interneurons from saline- ($1\pm 1\%$, $n=7$) and cocaine-exposed mice ($1\pm 1\%$, $n=7$), but it depolarized PLTS cells from saline-exposed mice ($5\pm 1\%$, $n=8$, $p=0.003$) and hyperpolarized PLTS cells from cocaine-exposed mice ($4\pm 1\%$, $n=6$; $p=0.02$, paired t -test; Fig 8E and F). While quinpirole reduced the extent of depolarization by bicuculline in saline-exposed FS (29% , $n=7$, $p=0.003$, t -test) and had little effect on quinpirole in PLTS interneurons (4% , $n=8$), quinpirole promoted substantial depolarization by bicuculline in both cocaine-exposed FS ($29\pm 8\%$, $p<0.001$) and PLTS interneurons ($24\pm 8\%$; $p=0.02$, paired t -test). Thus, the paradoxical reduction in GABA interneuron function following D2R activation was alleviated by the GABA_AR antagonist.

PCE also provoked abnormal excitatory, AMPA receptor-mediated responses in GABA interneurons. In saline-exposed mice, quinpirole had no effect on the eEPSC amplitude ($7\pm 10\%$; $n=11$; $p=0.4$) or the PPR ($0.5\pm 9\%$; $p=0.9$). The addition of bicuculline to quinpirole did not change the PPR ($-0.2\pm 10\%$), but enhanced cell excitability as the eEPSC amplitude increased ($55\pm 11\%$; $p<0.02$, compared with vehicle or quinpirole, paired t -test; Fig 8G). Following PCE, quinpirole reduced the eEPSC amplitude ($-18\pm 5\%$; $n=8$; $p=0.02$) and increased the PPR ($27\pm 7\%$; $p=0.01$). The addition of bicuculline to quinpirole increased the eEPSC amplitude ($29\pm 2\%$; $p=0.01$), while the PPR approached baseline ($-2\pm 5\%$; Fig 8H; $p=0.9$, compared with vehicle; $p=0.02$, compared to quinpirole, paired t -test).

Discussion

PCE can produce long-lasting behavioral and motor disturbances in affected children,^{4,5} and it reduced dopamine-dependent reflex and motor task performance in mice. These abnormal behaviors were paralleled by a long-lasting and reversible depression of corticostriatal activity that was generated through abnormally-functioning GABA interneurons (Fig 1B). PCE promoted inappropriate dopamine filtering of corticostriatal activity; rather than boosting stronger cortical connections by inhibiting the weak,¹⁵ D2Rs strengthened weaker glutamatergic synapses while stronger signals remained unperturbed (Fig 1).

GABA interneurons are created in the medial ganglionic eminence³⁶ and become potent regulators of corticostriatal signalling,³⁷ but their tangential migration is reduced following PCE.³⁸ PCE reduced FS and PLTS interneuron excitability and the downstream increase in GABA_BR1a-receptor subunit sensitivity would inhibit corticostriatal activity when extracellular GABA surges during synaptic activity.³⁹

While normally promoting inhibition at corticostriatal synapses,^{15,30} D2Rs boosted corticostriatal activity in cocaine-exposed mice. D2Rs likely reduced tonic inhibition of corticostriatal GABA_BRs by inhibiting excitatory inputs on GABA interneurons and by hyperpolarizing PLTS interneurons. Because only small currents are required to activate PLTS interneurons, minor decreases in the membrane potential become physiologically significant, requiring recruitment of additional excitatory inputs³⁹, which are also aberrantly controlled by D2Rs.

We also found that a GABA_AR antagonist can prevent this paradoxical excitation of corticostriatal activity by D2Rs. While GABA_AR blockade had no direct effect on corticostriatal activity,²⁹ it prevented the reduction in tonic GABA currents that occurred in response to D2R activation. Inhibition of GABA interneuron activity by GABA_A autoreceptors³³ is dependent on ambient GABA supplied by striatal neurons and inputs from the external pallidum.^{33,40,41} In controls, GABA_AR blockade suppressed inhibitory tonic GABA currents, the interneuron depolarized, but any increase in synaptic GABA remained undetected at corticostriatal GABA_BRs. PCE hyperpolarized GABA interneurons and also suppressed bicuculline-sensitive tonic GABA currents, which were reactivated by the D2R agonist. Thus, the combination of GABA_AR antagonist and D2R agonist would strengthen corticostriatal inhibition at GABA_BRs and oppose any reduction in tonic GABA caused by the D2R agonist alone.

D2Rs located on GABA interneurons³³ modulate tonic GABA currents through PKA³⁵ which is essential to the physiological states of both dopamine³⁰ and GABA_A receptors.^{14,34} In striatal MSNs, tonic GABA_AR currents are dependent on β 3-subunit phosphorylation which is inhibited by D2R inactivation of PKA through intracellular G_{i/o} protein coupling.³⁵ PCE down-regulates cortical β 3-GABA_AR subunits,²³ suggesting that these aberrant responses to D2R and GABA_AR activation may be due to alterations in receptor subunit expression, G-protein coupling, phosphorylation, or GABA transport.^{35,40}

Synaptic depression and dopamine-dependent excitation at corticostriatal synapses are also elicited by repeated psychostimulant exposure in adult mice.²⁰ In contrast to PCE, these phenomena in mature mice are produced through D1 and cholinergic receptors. PCE failed to elicit excitatory D1R responses, perhaps due to D1R uncoupling⁴² or because the dopamine-releasing effects of cocaine in rodents may not be possible until the first week of life.⁴³ Differences in plasticity were also reflected in the observed behaviors, as PCE reduced the magnitude of augmented locomotor responses to repeated amphetamine, but achieved comparable locomotion with controls in withdrawal. Thus, PCE did not spare the adaptive behavior that is linked to incentive saliency and drug dependence.⁴⁴

Other observations following PCE might also arise from paradoxical D2R responses generated by over-inhibited synapses. The reduction in body weight is consistent with dysregulated D2R-mediated hypophysiotrophic function⁴⁵ (as well as through reduced uteroplacental blood flow⁶) and decreased thermal reactivity suggests an abnormal D2R-dependent reflex arc.^{17,18} Cocaine-exposed mice also performed poorly on late rotarod trials and demonstrated a heightened response to aversive stimulation, suggesting that stress or unanticipated environmental changes cause inconsistent behaviors, similar to those reported in adolescent boys with a history of PCE.^{1,9}

These findings support and extend reports of abnormal GABA function in the cortex and hippocampus of mouse models for PCE.²³ Since GABA receptor ligands can reverse synaptic depression and allow appropriate D2R filtering at corticostriatal synapses, these findings provide insight into novel therapeutic approaches that may correct behaviors in cocaine-exposed mice and improve outcome in children with PCE, as well as a broad range of disorders that involve abnormal GABAergic signaling.⁴⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

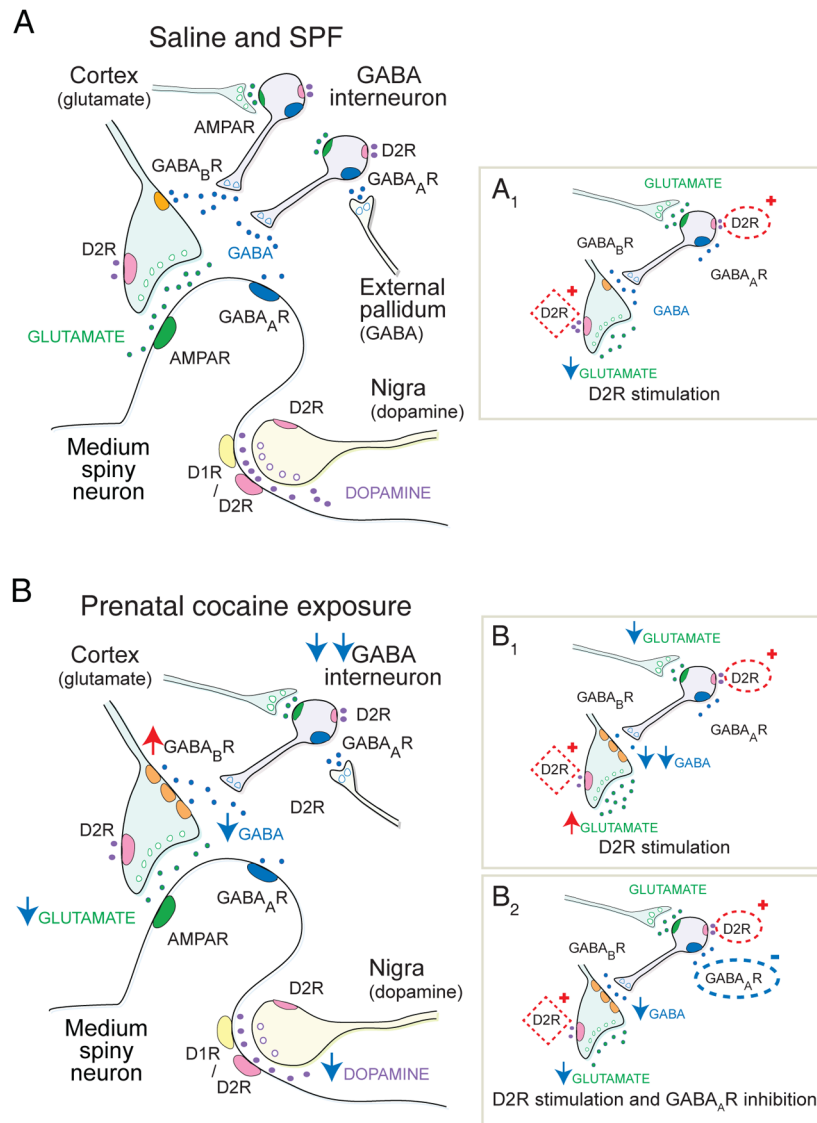
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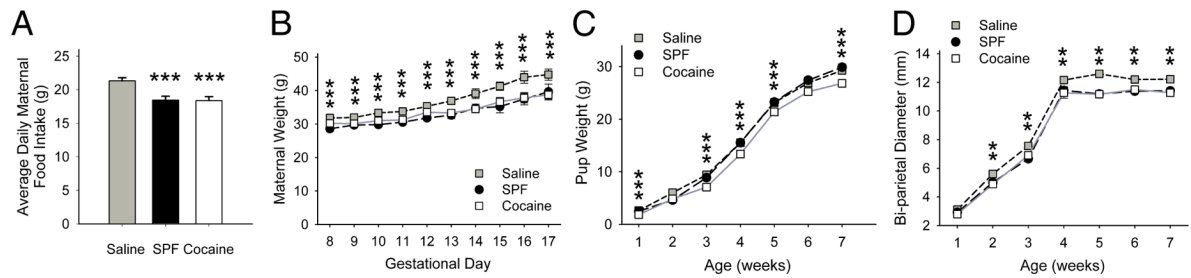
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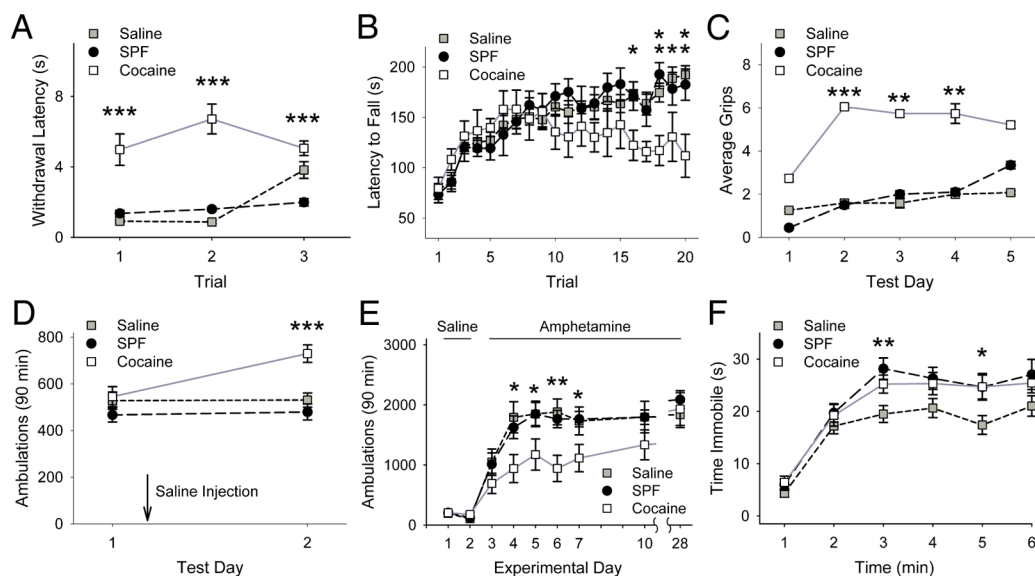
**FIGURE 1.**

The striatal ‘microcircuit’ and the proposed mechanism for over-inhibition of corticostriatal activity following PCE. (A) This simplified striatal circuit is composed of medium spiny neurons (MSNs) that are excited by glutamate and inhibited by dopamine and GABA. Glutamate (green) released from cortical afferents stimulates MSNs through post-synaptic AMPA receptors (R).³⁰ Dopamine (purple) modulates corticostriatal activity through D1- or D2-class dopamine receptors on MSNs and ‘filters’ corticostriatal activity through D2Rs acting on corticostriatal terminals.¹⁶ Synaptic ‘filtering’ occurs when dopamine inhibits a subset of cortical terminals with a low probability of release.^{12,16} GABA (blue) is capable of providing strong inhibition of corticostriatal activity through ionotropic GABA_ARs on MSNs, and also through metabotropic GABA_BRs located on corticostriatal terminals. GABA_BRs are relatively inactive in the resting state and provide little tonic inhibition.⁴¹ GABAergic fast-spiking and PLTS-type interneurons are excited by glutamate and are inhibited by dopamine D2Rs,³² as well as by GABA_A autoreceptors³³ that are tonically-activated by GABAergic inputs from the pallidum and other sources.⁴¹ (A₁) In saline and SPF mice, D2R stimulation with an agonist inhibits glutamate release from a subset of

cortical terminals (red box). D2R stimulation slightly depolarizes (activates) PLTS interneurons (red oval), but provides no downstream modulation of corticostriatal activity via GABA_BRs on cortical terminals. (B) PCE reduces GABA interneuron migration³⁸ and hyperpolarizes (inhibits) both FS and PLTS-type interneurons (double blue arrow). The putative reduction in GABA availability promotes overexpression of GABA_BR1a-receptor subunits which sensitizes GABA_BRs on corticostriatal terminals (red arrow) to produce tonic over-inhibition of glutamate release (blue arrow). Phasic dopamine release is also suppressed. (B₁) Stimulation of D2Rs (red oval) suppresses GABA interneurons, by reducing glutamate release from cortical afferents (blue arrow) and by hyperpolarizing PLTS-type GABA interneurons. The reduction in GABA availability (double blue arrow) relieves tonic inhibition at GABA_BRs on corticostriatal terminals to produce a paradoxical increase in glutamate release (red arrow). D2Rs located on corticostriatal terminals (red box) remain inhibitory, but since GABA_BRs are more potent modulators of presynaptic release,³⁰ corticostriatal activity is dominated by the change in GABA. (B₂) Blockade of inhibitory GABA_A autoreceptors (blue oval) has little effect on interneuron function following PCE, but competes with the D2R-dependent reduction in GABA inhibition (red oval) and prevents dopamine-dependent corticostriatal excitation following PCE. Therefore, when D2R are stimulated in the presence of a GABA_AR antagonist, the synapse may remain suppressed by tonic inhibition at GABA_BRs, but dopamine filtering (red box) is restored.

**FIGURE 2.**

PCE reduces growth. (A) Cocaine (18.38 ± 0.6 g/d) and SPF (18.44 ± 0.6 g/d) dams consumed less food than saline dams (21.33 ± 0.5 g/d). For panels A, B and D, ** $p < 0.01$, *** $p < 0.001$ for saline compared to SPF or cocaine, t -test. (B) The weight of cocaine-treated and SPF dams were similar throughout pregnancy and both weighed less than saline-exposed dams. (C) Cocaine pups weighed less than saline pups through week 6 and weighed less than SPF pups through week 7. SPF pups weighed less than saline pups on weeks 1 and 2 ($p < 0.05$, t -test), but weighed more than saline pups after week 3 ($p < 0.01$, t -test). (D) Saline pups had a larger cranial (bi-parietal) diameter than either cocaine or SPF pups after week 1 and there was no difference in the cranial diameter of cocaine and SPF mice ($p > 0.1$ t -test).

**FIGURE 3.**

PCE causes abnormal behaviors. (A) The latency for removing the tail from warm water was higher in cocaine-exposed mice over three trials, while the flick-latencies of saline and SPF mice were similar ($p=0.48$, Mann-Whitney). For panels A and C, $**p<0.01$, $***p<0.001$ for cocaine compared with saline or SPF, Mann-Whitney test. (B) Saline, SPF and cocaine-exposed mice spent a similar amount of time on the rotarod over the first 15 trials ($p=0.1$, t -test), but the falling latency decreased in cocaine mice after trial 15, while latencies of saline and SPF mice increased. For panels B, D, and E, $*p<0.05$, $**p<0.01$, $***p<0.001$ for cocaine compared with either saline or SPF mice, t -test. (C) Instead of falling, some mice would grip onto, and rotate with the rod. Cocaine mice gripped the rotarod more than saline or SPF mice, while there was no difference between the SPF and saline control groups ($p=0.1$, Mann-Whitney). (D) Open-field ambulations of saline, SPF and cocaine-exposed mice were similar on test day 1. A saline injection, administered immediately following the first test increased ambulations in cocaine-exposed mice on test day 2. (E) Saline, SPF, and cocaine mice were treated with saline for 2 days and then received amphetamine (2 mg/kg, i.p.) for 5 consecutive days. Mice were later challenged with amphetamine on days 10 and 28. Locomotor activity measured for 90 min following each amphetamine treatment revealed no stereotypic behaviors (data not shown). Locomotor activity increased in response to repeated amphetamine in all treatment groups. Ambulations in saline and SPF mice remained similar throughout testing ($p=0.07$, repeated-measures ANOVA). Cocaine-exposed mice demonstrated reduced ambulations on experiment days 4–7, but not following a drug challenge in withdrawal (days 10 and 28), as locomotor activity was similar in all groups ($p=0.1$, ANOVA). (F) When suspended by the tail, saline, SPF, and cocaine-exposed mice showed an equivalent increase in immobility over time, as there was no significant Treatment*Time effect ($p=0.16$, two-way ANOVA). These groups of mice also spent a similar amount of time immobile at most time points during the test (min 1, 2, 4, and 6). However, compared to saline, both cocaine and SPF mice spent a longer time immobile during min 3 and min 5. $*p=0.03$ and $**p=0.01$ for saline compared to either SPF or cocaine mice, t -test.

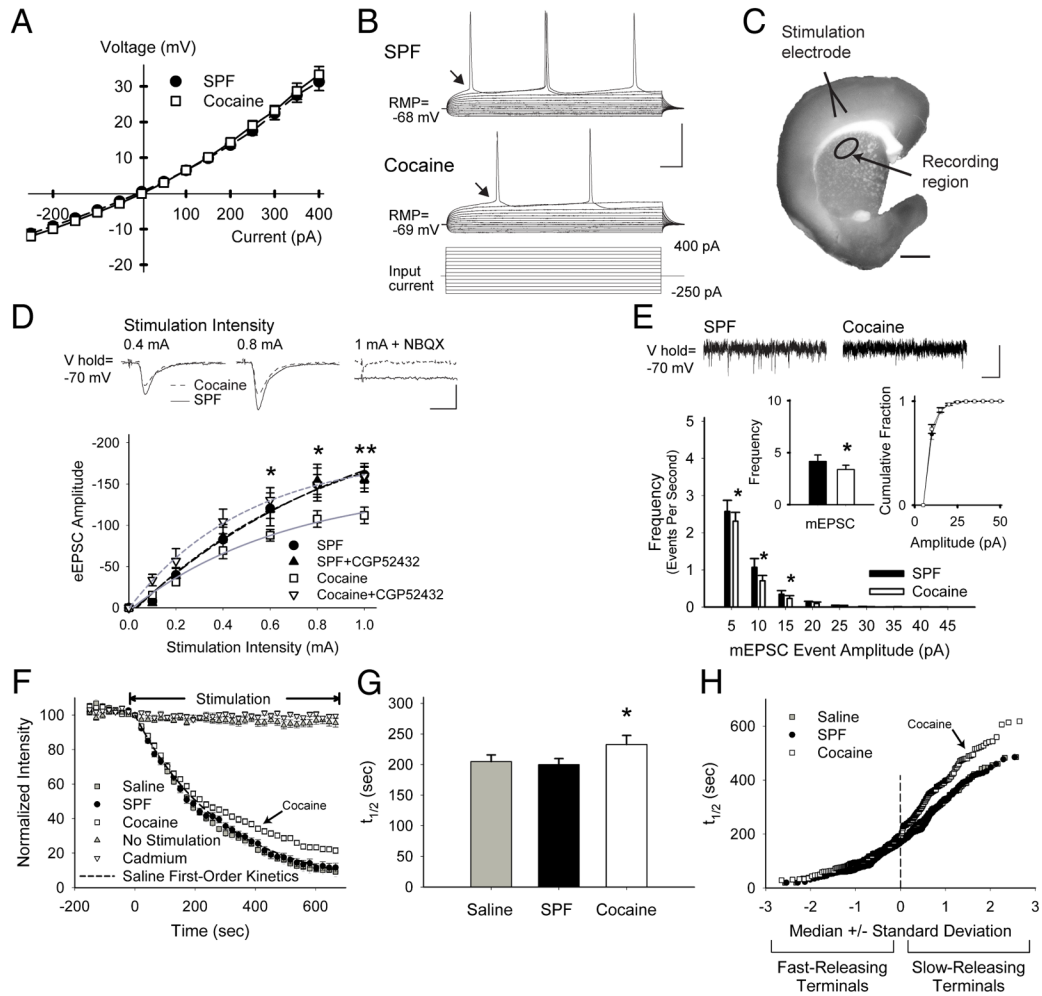
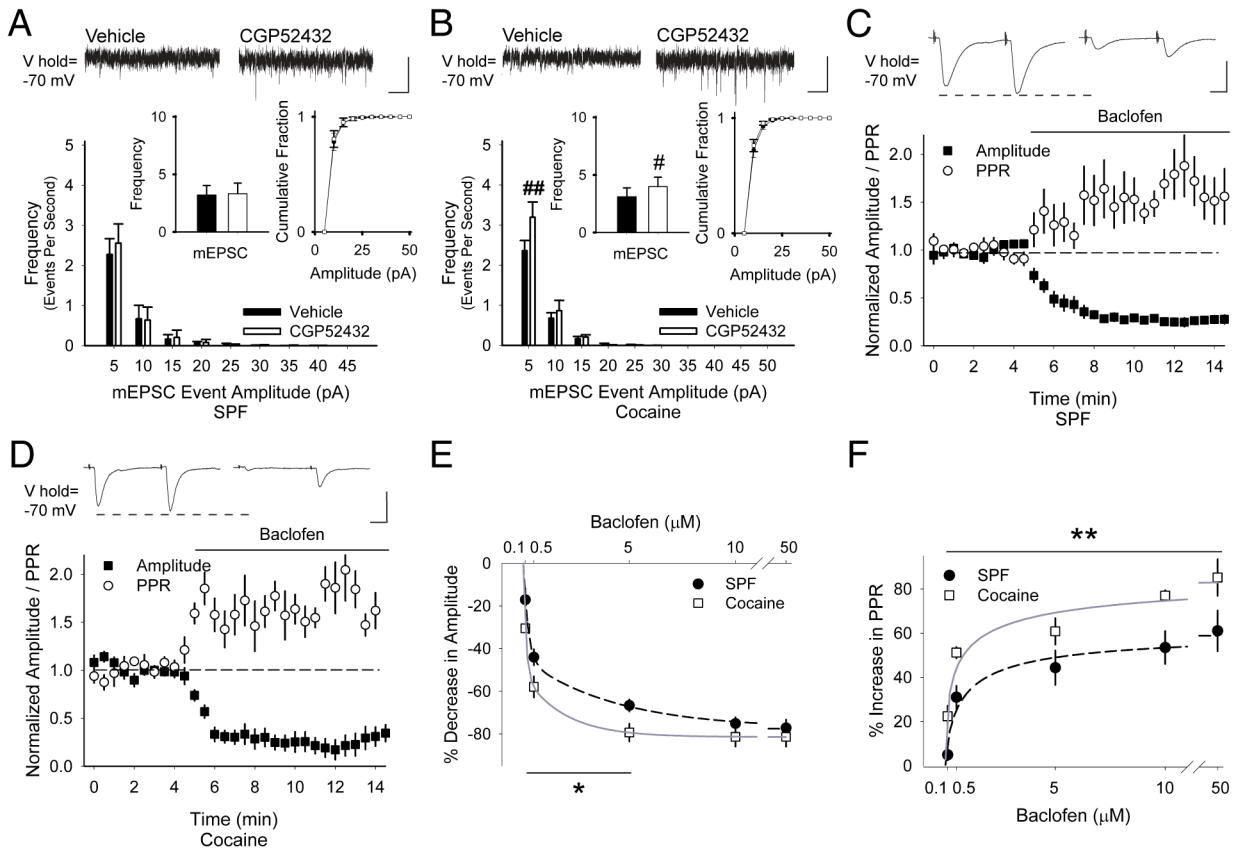


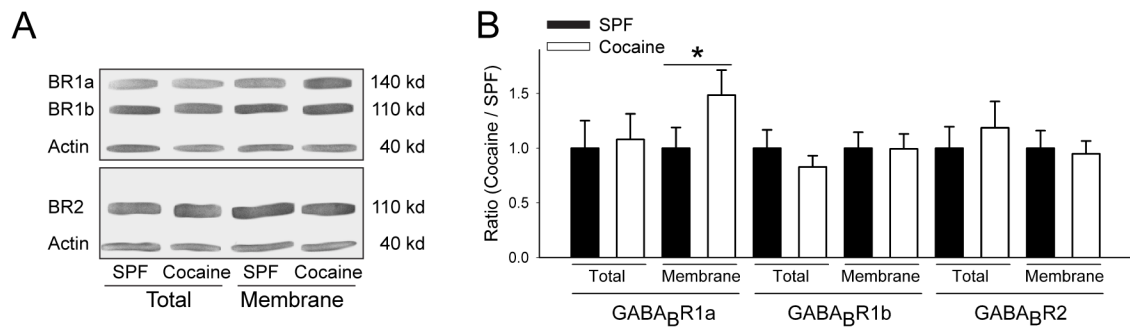
FIGURE 4.

PCE causes presynaptic depression through GABA_BRs. (A) Current clamp recordings in MSNs from SPF (n=15) and cocaine (n=11) mice displayed similar current-voltage curves with inward rectification, typical for MSNs (responses were measured at arrows in panel B). (B) Representative traces (above) demonstrate that fewer action potentials were generated in cells from cocaine mice in response to depolarizing current pulses (below). (C) The corticostriatal slice stained with FM1-43 and diaminobenzidine shows the areas of stimulation and recording. Corticostriatal activity was provoked using a bipolar stimulating electrode placed over cortical layers V–VI. Electrophysiological, optical, and biochemical recordings were obtained from the corresponding motor striatum (recording region), located 1.5–2.0 mm from the site of stimulation. (D) Representative traces (above) of voltage-clamp recordings show that similar cortical stimulation intensities evoked lower amplitude currents in MSNs from cocaine-exposed mice, compared to SPF. The AMPAR antagonist NBQX prevented evoked currents. Graph (below) shows the mean peak current evoked by the series of increasing cortical stimulation intensities in MSNs from SPF (n=10) and cocaine (n=12) mice. The GABA_BR antagonist CGP52432 had no effect on SPF cells, but blocked the reduction in eEPSC amplitudes in cocaine MSNs. * $p < 0.05$, ** $p < 0.01$, t -test. Cells were voltage clamped at -70 mV to minimize post-synaptic GABA_AR-mediated conductances (calculated $E_{Cl^-} = -74.2$ mV).⁴⁷ (E) Representative traces of mEPSCs (above; recorded in the presence of tetrodotoxin ($1\mu\text{M}$)) show a reduction in low-release probability (5–20 pA)

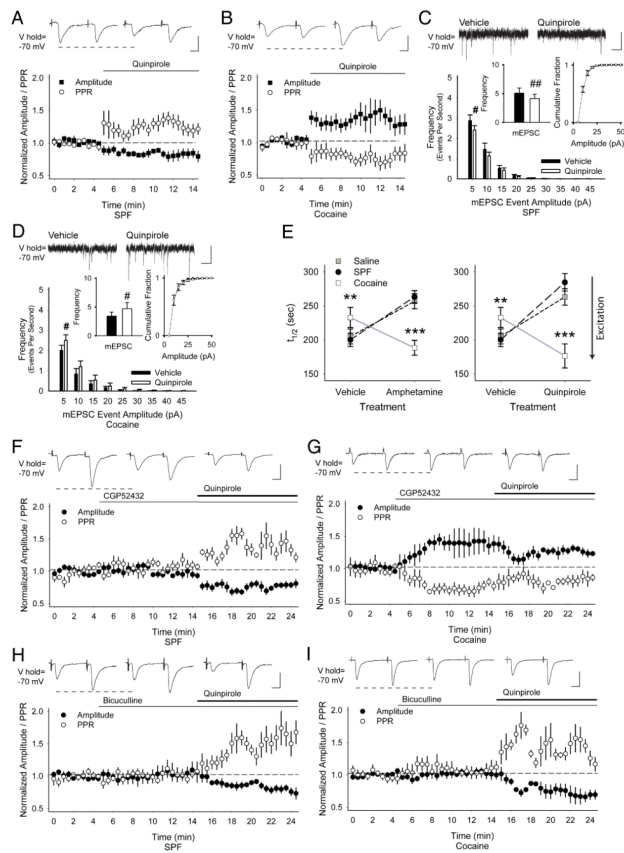
inward currents in cells from cocaine mice. The average frequency of mEPSCs (inset, left) was lower in cocaine MSNs (3.4 ± 0.4 Hz), compared to SPF (4.2 ± 0.6 Hz), while the cumulative mEPSC amplitude distributions (inset, right) were similar. $*p < 0.05$, *t*-test. (F) Stimulation of axons or cell bodies of projection neurons in layers V–VI of the cortex overlying the motor striatum resulted in endocytosis of FM1-43 dye by recycling synaptic vesicles, characteristic of corticostriatal afferents.¹⁶ Following dye loading, cortical stimulation at 20 Hz (beginning at $t=0$) resulted in exocytosis of FM1-43 dye from the terminals, which decreased in a manner approximated by a single exponent, characteristic of synaptic vesicle fusion.²² Feedback from MSNs was prevented using glutamatergic receptor antagonists (see Supplemental Methods). FM1-43 destaining was activity and calcium-dependent since no stimulation ($n=30$) or bath-applied cadmium ($200\mu\text{M}$; $n=25$) prevented stimulated release of the dye from presynaptic terminals. As FM1-43 destaining generally followed first-order kinetics, corticostriatal release was characterized by the half-time ($t_{1/2}$) of release, defined as the time required for terminal fluorescence to decay to half its initial value.²² (G) Mean \pm SEM halftimes of FM1-43 release for destaining curves shown in panel F. FM1-43 destaining was similar in slices from saline ($t_{1/2}=205$ sec) and SPF ($t_{1/2}=200$ sec) mice ($p=0.6$, Mann-Whitney), but was reduced in slices from cocaine mice ($t_{1/2}=233$ sec; $*p < 0.05$, Mann-Whitney). (H) An advantage of this optical technique is that we are able to examine vesicular release kinetics from individual cortical terminals. When the halftimes of individual terminals are presented relative to their standard deviation from the median value, a straight line indicates a normally-distributed (or single) population.¹⁵ Normal probability plots of individual terminal halftimes of release for experiments in panel F show that PCE decreased exocytosis from the slowest-releasing terminals (those with the highest $t_{1/2}$). This depression in corticostriatal release following PCE was not due to an inadequate innervation since the number of active corticostriatal terminals was higher in cocaine mice (61.4 ± 10 puncta vs. 41.9 ± 5 for saline; $p=0.02$, ANOVA). Bars: B, 40 mV, 25 ms; C, 1 mm; D, 50 pA, 5 ms; E, 10 pA, 250 ms. Curves were fit with a Hill equation.

**FIGURE 5.**

(A) Representative traces show that CGP52432 had no effect on the frequency of mEPSC in cells from SPF mice (3.2 ± 0.8 Hz in vehicle vs. 3.3 ± 0.9 Hz following CGP52432; $p=0.1$, paired t -test) and the cumulative distribution of mEPSC amplitudes was unchanged. (B) CGP52432 increased the frequency of mEPSCs in cells from cocaine mice (3.1 ± 0.8 Hz in vehicle vs. 3.9 ± 0.8 Hz following CGP52432) and the cumulative mEPSC amplitude distribution was unchanged. $\#p < 0.05$, $\#\#p < 0.01$, paired t -test. (C) Representative traces show the average responses to paired-pulses delivered at 50 ms every 30 sec before (above, left) and 5 to 7.5 min following bath application of baclofen (above, right). Graph shows the normalized amplitude of the first eEPSC (of the pair) and the normalized PPR. Baclofen reduced the amplitude of the first eEPSC (-92 ± 23 pA for vehicle vs. -21 ± 5 pA following baclofen) and increased the PPR (1.1 ± 0.1 in vehicle to 1.6 ± 0.2 following baclofen) in MSNs from SPF mice. (D) In cells from cocaine-exposed mice, baclofen reduced the amplitude of the first eEPSC (-59 ± 6 pA for vehicle vs. -13 ± 4 pA in baclofen) and increased the PPR (1.2 ± 0.1 in vehicle vs. 1.8 ± 0.4 in baclofen). (E) Concentration-curves demonstrate that baclofen (100 nM – 50 μ M) reduced the amplitude of the first eEPSC and (F) increased the PPR to a greater degree in MSNs from cocaine-exposed mice than in SPF controls ($IC_{50} = 4.2$ μ M;⁴⁸ $n=6, 5, 12, 5, 6$ cells for SPF and $n=4, 5, 5, 4$ for cocaine. $*p < 0.05$, $**p < 0.01$, ANOVA). Bars: A and B, 10 pA, 250 ms; C and D, 100 pA, 12.5 ms.

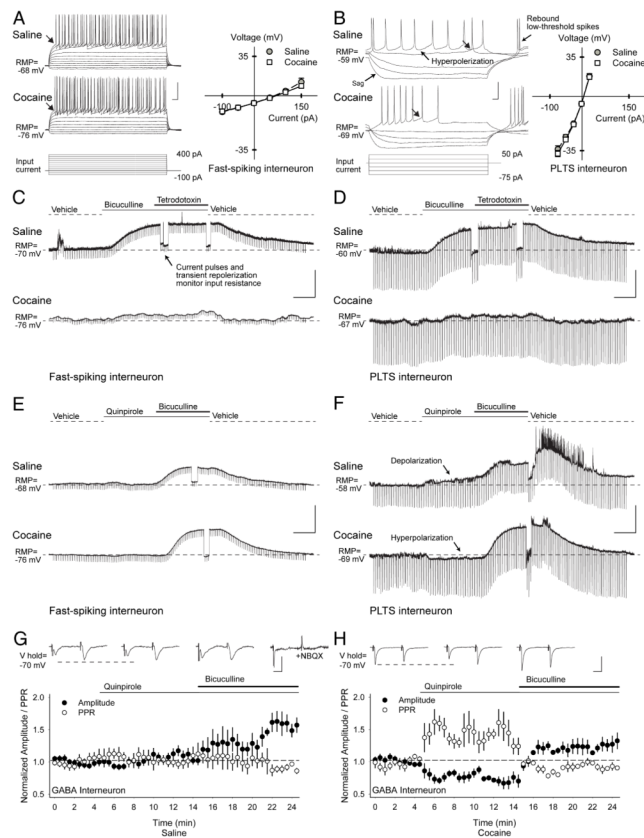
**FIGURE 6.**

PCE increases the surface expression of GABA_BR1a receptor subunits in the dorsal striatum. (A) Representative immunoblots of lysates from the dorsal striatum of 60-day-old SPF and cocaine-exposed mice compare levels of total and surface membrane-bound fractions of GABA_BR1, GABA_BR2, and actin. Antibody against GABA_BR1 labeled 2 bands, GABA_BR1a and GABA_BR1b. (B) Densitometry measurements of receptor protein bands presented as the ratio of densities for SPF and cocaine-exposed mice measured in the same gel (* $p < 0.05$, t -test).

**FIGURE 7.**

D2Rs provoke GABA_AR-dependent paradoxical responses following PCE. (A) In MSNs from SPF mice, representative traces (above) and graph demonstrate that the D2R agonist quinpirole reduces the amplitude of the first eEPSC (in each pair; -160 ± 27 pA for vehicle vs. -140 ± 29 pA following quinpirole) and increases the PPR (1.2 ± 0.1 in vehicle to 1.6 ± 0.2 in quinpirole). (B) In MSNs from cocaine mice, quinpirole increased the amplitude of the first eEPSC (-81 ± 18 pA for vehicle vs. -106 ± 20 pA for quinpirole) and the PPR decreased (1.4 ± 0.2 in vehicle vs. 1.0 ± 0.1 in quinpirole). (C) In MSNs from SPF mice, quinpirole diminished the frequency (inset, left; 5.1 ± 0.9 Hz in Veh vs. 4.1 ± 0.8 Hz in quinpirole), but not amplitude (inset, right) of low-release probability (5–10 pA) inward currents. For panels C and D, $\#p < 0.05$, $\#\#p < 0.01$, paired *t*-test. (D) In MSNs from cocaine-exposed mice, quinpirole increased the frequency (3.4 ± 0.7 Hz in Veh vs. 4.7 ± 1 Hz in quinpirole) of low-release probability mEPSCs, while having no effect on the cumulative amplitude distribution. (E) Amphetamine (left) and quinpirole (right) decreased FM1-43 destaining in slices from saline and SPF mice, but increased release in slices from cocaine mice. $*p < 0.05$, $**p < 0.01$, Mann-Whitney. (F) In cells from SPF mice, the GABA_BR antagonist CGP52432 did not change the eEPSC amplitude (-132 ± 20 pA for Veh vs. -122 ± 16 pA for CGP52432) or the PPR (1.3 ± 0.1 in Veh vs. 1.4 ± 0.1 in CGP52432). When quinpirole was added to CGP52432, the eEPSC amplitude decreased (-115 ± 15 pA) and the PPR increased (1.6 ± 0.1). (G) In MSNs from cocaine-exposed mice, the GABA_BR antagonist increased the eEPSC amplitude (-94 ± 23 pA for Veh vs. -121 ± 26 pA for CGP52432) and decreased the PPR (1.5 ± 0.1 in Veh vs. 1.0 ± 0.1 in CGP52432). When quinpirole was added to CGP52432, there was a slight reduction in eEPSC amplitude (-112 ± 12 pA) and an increase in the PPR (1.3 ± 0.1). (H) In MSNs from SPF mice, bicuculline did not change the amplitude of the first evoked current (-143 ± 31 pA for Veh vs. -148 ± 33 pA for bicuculline; $p = 0.1$) or the PPR

(1.1 ± 0.1 in Veh vs. 1.2 ± 0.2 in bicuculline; $p=0.06$, paired t -test). When quinpirole was added to bicuculline, the eEPSC amplitude decreased (-132 ± 22 pA) and the PPR increased (1.5 ± 0.1). (I) In MSNs from cocaine mice, bicuculline did not change the amplitude of the eEPSC (-103 ± 24 pA for Veh vs. -101 ± 21 pA for bicuculline) or the PPR (0.9 ± 0.1 in Veh vs. 0.9 ± 0.1 in bicuculline). When quinpirole was added to bicuculline, the eEPSC amplitude decreased (-82 ± 21 pA) and the PPR increased (1.3 ± 0.1). Bars: A, B, F, and G–I, 100 pA, 12.5 ms; C and D, 10 pA, 250 ms.

**FIGURE 8.**

Abnormal GABA interneuron function. (A) Current clamp recordings show characteristic responses of FS and (B) PLTS interneurons from *Lhx6*-GFP transgenic mice to hyperpolarizing and depolarizing current injections (below). Both FS and PLTS interneurons from cocaine cells had lower resting membrane potentials (RMP) than saline cells. Current-voltage plots (right) show similar responses in saline and cocaine-exposed cells after subtraction of their resting membrane potentials (responses were measured at arrows). FS cells were silent at rest, and displayed a high firing rate with little adaptation following depolarizing current injection. Spikes were short and followed by a large after-hyperpolarization. PLTS interneurons exhibited a marked time-dependent sag in response to hyperpolarizing current injections and a rebound persistent low-threshold spike and/or a plateau potential persisted after termination of hyperpolarizing current. During current injections, both FS and PLTS interneurons displayed a variable pattern of spike bursts (1–48 action potentials) interspersed by membrane oscillations. Compared to FS interneurons, PLTS interneurons exhibited a much higher input resistance, a lower resting membrane potential (RMP), and a much lower input current was required to produce action potentials (Supplementary Tables S3–S5), with values similar to those reported previously.^{14,49} (C) Representative current-clamp recordings in FS and (D) PLTS interneurons from saline- (above) and cocaine-exposed mice (below) demonstrate typical responses to the GABA_AR antagonist bicuculline before and after bath application of the sodium channel blocker tetrodotoxin. In FS and PLTS interneurons, bicuculline depolarized saline-exposed cells (the RMP became more positive) to a much greater degree than cocaine-exposed cells. GABA likely produced tonic inhibition at GABA_A autoreceptors¹⁴ since the change in membrane potential by bicuculline in saline-exposed mice (FS, $37 \pm 2\%$, $p < 0.001$; PLTS, $23 \pm 7\%$, $p = 0.03$) and cocaine-exposed mice (FS, $3 \pm 1\%$, $p = 0.09$; PLTS, $4 \pm 1\%$, $p = 0.003$, paired *t*-test)

persisted when synaptic transmission was blocked by tetrodotoxin. Note that the cellular input resistance was monitored by (250 ms, 100 pA) current pulses applied every 10 sec. Changes in input resistance during depolarization were measured after transiently repolarizing the cell to resting membrane potential levels. Interestingly, bicuculline reduced the input resistance (Supplementary Fig S5), suggesting recruitment of additional ion channels with depolarization that are critical for sustained high-frequency firing.³⁹ (E) Representative current-clamp recordings in FS and (F) PLTS interneurons from saline- (above) and cocaine-exposed mice (below) demonstrate typical responses to the D2 receptor agonist quinpirole before and after bicuculline. Quinpirole had no effect in FS interneurons, but slightly depolarized saline-exposed PLTS cells, while hyperpolarizing cocaine-exposed PLTS cells. For all interneurons, the membrane potential became more positive and the cell depolarized when bicuculline was added to quinpirole. A summary of membrane potentials and input resistance for FS and PLTS interneurons under all conditions tested can be found in Supplementary Fig S5. (G) Excitatory inputs onto GFP+ fluorescent interneurons from Lhx6-GFP transgenic mice were activated with paired-pulses using cortical bipolar stimulating electrodes. The PPR was similar in cells from saline (1.35 ± 0.11 ; $n=11$) and cocaine-exposed mice (1.13 ± 0.09 ; $n=8$; $p=0.2$, t -test). In saline-exposed mice, representative traces (above) and graph show that quinpirole did not change the amplitude of the first eEPSC (-32 ± 3 pA in vehicle vs. -35 ± 6 pA following quinpirole) or the PPR (1.3 ± 0.1 in vehicle vs. 1.3 ± 0.1 in quinpirole). When quinpirole was combined with bicuculline, the eEPSC amplitude increased (-45 ± 6 pA), but the PPR remained unchanged (1.3 ± 0.3). The AMPA-receptor antagonist NBQX ($10 \mu\text{M}$) abolished the eEPSC. (H) In GFP+ interneurons from cocaine-exposed mice, quinpirole reduced the amplitude of the first eEPSC (-62 ± 5 pA in vehicle vs. -49 ± 9 pA following quinpirole) and the PPR increased (1.1 ± 0.1 in vehicle vs. 1.4 ± 0.2 in quinpirole). Paired-pulse depression was observed in the presence of bicuculline, which prevented the change in PPR (1.1 ± 0.2) and the eEPSC amplitude increased (-95 ± 13 pA). Bars: A and B, 30 mV, 20 ms; C–F, 30 mV, 2 min; G and H, 50 pA, 12.5 ms.