Overinhibition of Corticostriatal Activity following Prenatal Cocaine Exposure

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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 polysubstance 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 after repeated treatment with amphetamine. These abnormal behaviors were consistent with abnormal γ -aminobutyric acid (GABA) interneuron function, which promoted a reversible depression in corticostriatal activity. PCE hyperpolarized and reduced tonic GABA currents in both fast-spiking and persistent low-threshold spiking type GABA interneurons to increase tonic inhibition at GABA_B receptors on presynaptic corticostriatal terminals. Although D2 receptors paradoxically increased glutamate release following PCE, normal corticostriatal modulation by dopamine was reestablished with a GABA_A receptor 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 GABAergic mechanisms. Our results indicate approaches that 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. ANN NEUROL 2013;73:355–369

Prenatal cocaine exposure (PCE) can cause debilitating neuropsychological and motor abnormalities in humans and is an important public health concern.¹ Despite prevention-related measures, >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 γ -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

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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), C57Bl6 strain (n = 9), and Lhx6-GFP BAC transgenic (n = 10; MMRRC-GENSAT project) dams received either cocaine (20mg/kg subcutaneously) or saline twice daily from embryonic day 8 through 18.⁷ As cocaine causes anorexia,⁷ controls included a saline-treated, pair-fed (SPF) group whose nutritional intake was matched to that of cocaine-treated dams, and a saline-treated group that was 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 to 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 to 32 and 55 to 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 to 33 days and 60 to 80 days, as described¹⁶ in the Supplementary Methods.

Western Blotting

Tissue was extracted from the dorsal striatum of 14 mice, aged 63 to 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 subsecond resolution in striatal slices from 14 mice, aged 63 to 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 \pm standard error of the mean. Differences, considered significant if p < 0.05, were assessed with appropriate *t* tests, analyses of variance (ANOVAs), or the nonparametric Mann–Whitney test (see Supplementary Methods).

Results

Abnormal Growth and Dopamine-Dependent Behaviors

Timed-pregnant cocaine-exposed (n = 19) and SPF Swiss Webster dams (n = 17) consumed similar quantities of food throughout gravidity, but 14% less than saline dams (n = 20; p < 0.001, t test; Fig 2). 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). 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), and 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, ttest). Thus, the reduction in postnatal weight was dependent on PCE, whereas 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 (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 cocaineexposed mice (n = 8; p < 0.001, repeated-measures ANOVA; see 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, ANOVA) and cocaine mice gripped the rod

FIGURE 1: The striatal microcircuit and the proposed mechanism for overinhibition of corticostriatal activity following prenatal cocaine exposure (PCE). (A) This simplified striatal circuit is composed of medium spiny neurons (MSNs) that are excited by glutamate and inhibited by dopamine and y-aminobutyric acid (GABA). Glutamate (green) released from cortical afferents stimulates MSNs through post-synaptic α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (Rs).³⁰ Dopamine (purple) modulates corticostriatal activity through D1 or D2 class dopamine receptors on MSNs and filters corticostriatal activity though 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 GABAARs on MSNs, and also through metabotropic GABARs located on corticostriatal terminals. GABARs are relatively inactive in the resting state and provide little tonic inhibition.⁴¹ GABAergic fast-spiking and persistent low-threshold spiking (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 (A_1) In saline and saline-treated, pair-fed (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 fast spiking and PLTS type interneurons (double blue arrows). The putative reduction in GABA availability promotes overexpression of GABA_RR1a subunits, which sensitizes GABA_RRs on corticostriatal terminals (red arrow) to produce tonic overinhibition 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 arrows) 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 because GABA_BRs are more potent modulators of presynaptic release,³⁰ corticostriatal activity is dominated by the change in GABA. (B2) Blockade of inhibitory GABAA 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 D2Rs 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: Prenatal cocaine exposure reduces growth. (A) Cocaine (18.38 \pm 0.6g/d) and saline-treated, pair-fed (SPF; 18.44 \pm 0.6g/d) dams consumed similar quantities of food throughout gravidity (p = 0.95), but less food than saline dams (21.33 \pm 0.5g/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 was 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 (***p < 0.001, cocaine compared to either saline or SPF, t test). 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 (biparietal) 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: Prenatal cocaine exposure causes abnormal behaviors. (A) The latency for removing the tail from warm water was higher in cocaine-exposed mice over 3 trials, whereas the flick latencies of saline and saline-treated, pair-fed (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, whereas 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 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, whereas there was no difference between the SPF and saline control groups (p = 0.1, Mann-Whitney). (D) Open-field ambulation of saline, SPF, and cocaine-exposed mice was similar on test day 1. A saline injection, administered immediately following the first test, increased ambulation 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 (2mg/kg intraperitoneally) for 5 consecutive days. Mice were later challenged with amphetamine on days 10 and 28. Locomotor activity measured for 90 minutes following each amphetamine treatment revealed no stereotypic behaviors (data not shown). Locomotor activity increased in response to repeated amphetamine in all treatment groups. Ambulation in saline and SPF mice remained similar throughout testing (p = 0.07, repeated-measures analysis of variance [ANOVA]). Cocaine-exposed mice demonstrated reduced ambulation 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 cocaineexposed mice showed an equivalent increase in immobility over time, as there was no significant treatment \times time effect (p = 0.16, 2-way ANOVA). These groups of mice also spent a similar amount of time immobile at most time points during the test (minutes 1, 2, 4, and 6). However, compared to saline, both cocaine-exposed and SPF mice spent a longer time immobile during minute 3 and minute 5. *p = 0.03 and **p = 0.01 for saline compared to either SPF or cocaine mice, t test.

more frequently than either saline or SPF mice (p < 0.001, Mann–Whitney; see Fig 3C).

Locomotion in the open field was similar in saline (n = 35), SPF (n = 38), and cocaine-exposed mice (n = 24; see Fig 3D). Following a saline injection, the ambulation of saline and SPF mice remained similar, whereas 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 intraperitoneally). 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), but comparable sensitized responses to amphetamine challenges in withdrawal (see Fig 3E).

As a control experiment to test for hypodopaminergia²¹ and depression,²⁶ mice were suspended by the tail, and time spent immobile was measured. Whereas there was no overall difference between saline (n = 49), SPF (n = 29), and cocaine mice (n = 29; see Fig 3F), both SPF and cocaine-exposed mice displayed small increases in immobility compared to saline, 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 4 and Supplementary Table S1). However, compared to SPF mice, MSNs from cocaine-exposed mice were 36% less responsive to current injection (p < 0.001, ANOVA), and cortical stimulation >0.6mA evoked currents lower-amplitude excitatory postsynaptic (eEPSCs; p < 0.05, 2-way ANOVA). This inhibition of corticostriatal activity following PCE was likely of presynaptic origin,²⁸ because the eEPSC paired-pulse ratio (PPR) was higher in cocaine (1.2 \pm 0.05; n = 49 cells) than in SPF (1.07 \pm 0.03; n = 60; p = 0.01, t test; not shown), and the frequency of miniature EPSCs (mEPSCs) was 18% less in cocaine-exposed cells (n = 19) compared to SPF (n = 14; p = 0.03, t test), whereas the cumulative mEPSC amplitude distributions were unchanged.

Exocytosis from corticostriatal terminals was directly examined by multiphoton microscopy using the endocytic tracer FM1-43.¹⁵ The half-time 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, whereas the faster-releasing terminals remained unperturbed (see Fig 4F–H). This synaptic depression was long-lasting, because exocytosis in younger 30-day-old mice was 18% lower in cocaine-exposed mice (t_{1/2} = 245 seconds; n = 114) compared to saline (t_{1/2} = 201 seconds; n = 68; p = 0.01, Mann–Whitney; not shown).

GABA_BR-Dependent Overinhibition 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

FIGURE 4: Prenatal cocaine exposure (PCE) causes presynaptic depression through γ-aminobutyric acid (GABA)_B receptors (Rs). (A) Current clamp recordings in medium spiny neurons (MSNs) from saline-treated, pair-fed (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 hyperpolarizing and depolarizing input currents (below). RMP = resting membrane potential. (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 to 2.0mm 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 α-amino-3-hydroxy-5methylisoxazole-4-propionic acid receptor antagonist NBQX (10µM) prevented evoked currents, indicating that evoked currents were glutamatergic. 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 evoked excitatory postsynaptic current (eEPSC) amplitudes in cocaine MSNs. *p < 0.05, **p < 0.01, t test. Cells were voltage clamped at -70mV to minimize postsynaptic GABA_AR-mediated conductances (calculated $E_{cl}^{-} = -74.2 \text{mV}$).⁴⁷ (E) Representative traces of miniature excitatory postsynaptic currents (mEPSCs; above) recorded in the presence of tetrodotoxin (1 μ M) show a reduction in low release probability (5–20pA) inward currents in cells from cocaine mice. The average frequency of mEPSCs (inset, left) was lower in cocaine MSNs (3.4 ± 0.4Hz), compared to SPF (4.2 ± 0.6Hz), where 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 20Hz (beginning at time = 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 Supplementary Methods). FM1-43 destaining was activity- and calcium-dependent, because no stimulation (n = 30) or bath-applied cadmium (200μ 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 ± standard error of the mean half-times of FM1-43 release for destaining curves shown in panel F. FM1-43 destining was similar in slices from saline ($t_{1/2}$ = 205 seconds) and SPF ($t_{1/2}$ = 200 seconds) mice (p = 0.6, Mann–Whitney), but was reduced in slices from cocaine mice ($t_{1/2} = 233$ seconds; *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 half-times 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 half-times 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 inadequate innervation, because the number of active corticostriatal terminals was higher in cocaine mice (61.4 \pm 10 puncta vs 41.9 \pm 5 for saline; p = 0.02, analysis of variance). Bars: B, 40mV, 25 milliseconds; C, 1mm; D, 50pA, 5 milliseconds; E, 10pA, 250 milliseconds. Curves were fit with a Hill equation.



FIGURE 5: Prenatal cocaine exposure increases the sensitivity of y-aminobutyric acid_B receptors. (A) Representative traces show that CGP52432 had no effect on the frequency of miniature excitatory postsynaptic current (mEPSC) in cells from salinetreated, pair-fed (SPF) mice (3.2 \pm 0.8Hz in vehicle vs 3.3 \pm 0.9Hz 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 ± 0.8Hz in vehicle vs 3.9 ± 0.8Hz 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 milliseconds every 30 seconds before (above, left) and 5 to 7.5 minutes following bath application of baclofen (above, right). Graph shows the normalized amplitude of the first evoked excitatory postsynaptic current (eEPSC; of the pair) and the normalized paired-pulse ratio (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 \pm 0.1 in vehicle to 1.6 \pm 0.2 following baclofen) in medium spiny neurons (MSNs) from SPF mice. (D) In MSNs from cocaine-exposed mice, baclofen reduced the amplitude of the first eEPSC $(-59 \pm 6pA$ for vehicle vs $-13 \pm 4pA$ in baclofen) and increased the PPR (1.2 \pm 0.1 in vehicle vs 1.8 \pm 0.4 in baclofen). (E) Concentration curves demonstrate that baclofen (100nM-50µM) 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 ($IC_{50} = 4.2 \mu M^{48}$; n = 6, 5, 12, 5, 6 cells for SPF and n = 4, 5, 5, 4 cells for cocaine; *p < 0.05, **p < 0.01, analysis of variance). Bars: A and B, 10pA, 250 milliseconds; C and D, 100pA, 12.5 milliseconds.

Tonic inhibition by GABA_BRs was absent in SPF mice, because the GABA_BR antagonist CGP52432 (10 μ M) did not change the eEPSC amplitude (see Fig 4D) or the frequency of mEPSCs (10 ± 14%; n = 7 cells; Fig 5A). However, CGP52432 prevented the tonic inhibition of MSNs from cocaine-exposed mice (see Fig 4D) and increased the frequency of mEPSCs (19 ± 3%; n = 6; p = 0.02, paired *t* test; see Fig 5B).

To determine whether PCE might change the sensitivity of presynaptic GABA_BRs, eEPSCs from MSNs were measured in response to cortical stimulation with 50-millisecond paired pulses, applied every 30 seconds.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 ± 3%; p = 0.002), and the PPR increased (44 ± 8%; n = 14; p < 0.001, paired t test; see 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 ± 4%; n = 8; p =0.03), and the PPR increased (61 ± 6%; p = 0.01, paired t test; see 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; see Fig 5E, F), suggesting that PCE increases GABA_BR sensitivity.



FIGURE 6: Prenatal cocaine exposure increases the surface expression of γ -aminobutyric acid (GABA)_B receptor (R)1a subunits in the dorsal striatum. (A) Representative immunoblots of lysates from the dorsal striatum of saline-treated, pair-fed (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).

Overexpression of GABA_BR1a 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 subunit surface protein from cocaine-exposed striatum (1.41 \pm 0.3) compared to SPF (1 \pm 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 subunits, which are preferentially expressed on corticostriatal terminals.³¹

Paradoxical Excitation by D2Rs Is Dependent on $GABA_BRs$

Dopamine modulates corticostriatal activity through D2Rs,¹⁶ and in MSNs from SPF mice, the D2R agonist quinpirole decreased the amplitude of the first eEPSC $(-15 \pm 7\%; n = 9 \text{ cells}; p = 0.004)$ and increased the PPR (30 \pm 8%; p = 0.01, paired t test; Fig 7). 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). This aberrant excitation of corticostriatal activity by D2Rs following PCE was likely presynaptic, because quinpirole reduced the frequency of mEPSCs in SPF cells (-18 \pm 6%; n = 7; p = 0.003), but enhanced their frequency in cocaine-exposed neurons (38 \pm 14%; n = 8; p = 0.04, paired t test). 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 (see Supplementary Methods and Supplementary 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 (see Supplementary Methods and Supplementary Fig S2). However, optical experiments in slices from saline and SPF mice confirmed that both the dopamine releaser amphetamine¹⁵ (10μ M) and quinpirole reduced exocytosis by specifically modulating glutamatergic inputs with a low probability of release (see Fig 7E, Supplementary Methods, and Supplementary Fig S3), but boosted release from those same synapses in slices from cocaineexposed 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 (see Supplementary Methods and Supplementary Fig S4).

D2Rs inhibit corticostriatal release in untreated mice,¹⁵ and also suppress GABA interneuron function,³² and might increase corticostriatal activity by reducing tonic inhibition at sensitized GABA_BRs. In MSNs 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; see 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; see 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 < 0.05compared with vehicle or CGP52432, paired t test), possibly due to concurrent corticostriatal inhibition by D2Rs.³⁰



FIGURE 7:

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 ($10\mu M$) did not change the eEPSC amplitude (5 \pm 3%; n = 9) or the PPR (12 \pm 7%; see 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 the paradoxical excitation by D2Rs, because 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; see Fig 7I).

PCE Alters GABA Interneuron Function

Because results indicated that cocaine-induced corticostriatal plasticity might be 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; Fig 8A and B, Supplementary Tables S2–S4).

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; see Fig 8C, D). However, cocaine-exposed FS and PLTS interneurons 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 β 3-GABA_AR subunit expression following PCE.^{23,27}

D2Rs caused paradoxical corticostriatal excitation following PCE and can reduce tonic GABA inhibition through intracellular G-protein-coupled phosphorylation of GABAAR subunits by protein kinase A (PKA).³⁵ The D2R agonist quinpirole did not change the membrane potential in FS interneurons from saline-exposed (1 \pm 1%; n = 7) and cocaine-exposed mice (1 ± 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; see Fig 8E, F). Quinpirole reduced the extent of depolarization by bicuculline in saline-exposed FS interneurons (29%; n = 7; p = 0.003, t test) and had little effect on bicuculline depolarization in PLTS interneurons (4%; n = 8). However, 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).

FIGURE 7: D2Rs provoke γ-aminobutyric acid (GABA)_A receptor (R)-dependent paradoxical responses following prenatal cocaine exposure. (A) In medium spiny neurons (MSNs) from saline-treated, pair-fed (SPF) mice, representative traces (above) and graph demonstrate that the D2R agonist quinpirole reduces the amplitude of the first evoked excitatory postsynaptic current (eEPSC; in each pair; -160 ± 27pA for vehicle vs -140 ± 29pA following quinpirole) and increases the paired-pulse ratio (PPR; 1.2 ± 0.1 in vehicle vs 1.6 ± 0.2 in quinpirole). (B) In MSNs from cocaine mice, quinpirole increased the amplitude of the first eEPSC (-81 \pm 18pA for vehicle vs -106 \pm 20pA for quinpirole), and the PPR decreased (1.4 \pm 0.2 in vehicle vs 1.0 \pm 0.1 in quinpirole). (C) In MSNs from SPF mice, quinpirole diminished the frequency (inset, left; 5.1 ± 0.9Hz in vehicle vs 4.1 ± 0.8Hz in quinpirole), but not amplitude (inset, right) of low release probability (5-10pA) inward currents. For panels C and D, #p < 0.05, #p < 0.01, paired t test. (D) In MSNs from cocaine-exposed mice, guinpirole increased the frequency (3.4 \pm 0.7Hz in vehicle vs 4.7 ± 1Hz in quinpirole) of low release probability miniature excitatory postsynaptic currents (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 < 0.01, ***p < 0.001, cocaine compared to saline or SPF, Mann-Whitney. (F) In cells from SPF mice, the GABA_BR antagonist CGP52432 did not change the eEPSC amplitude (-132 \pm 20pA for vehicle vs -122 \pm 16pA for CGP52432) or the PPR (1.3 \pm 0.1 in vehicle vs 1.4 \pm 0.1 in CGP52432). When quinpirole was added to CGP52432, the eEPSC amplitude decreased (-115 \pm 15pA), and the PPR increased (1.6 \pm 0.1). (G) In MSNs from cocaine-exposed mice, the GABA_BR antagonist increased the eEPSC amplitude $(-94 \pm 23$ pA for vehicle vs -121 ± 26 pA for CGP52432) and decreased the PPR (1.5 \pm 0.1 in vehicle vs 1.0 \pm 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 \pm 0.1). (H) In MSNs from SPF mice, bicuculline did not change the amplitude of the first evoked current (-143 \pm 31pA for vehicle vs -148 \pm 33pA for bicuculline; p = 0.1) or the PPR (1.1 \pm 0.1 in vehicle vs 1.2 \pm 0.2 in bicuculline; p = 0.06, paired t test). When guinpirole was added to bicuculline, the eEPSC amplitude decreased (-132 ± 22pA), and the PPR increased (1.5 \pm 0.1). (I) In MSNs from cocaine mice, bicuculline did not change the amplitude of the eEPSC (-103 \pm 24pA for vehicle vs -101 \pm 21pA for bicuculline) or the PPR (0.9 \pm 0.1 in vehicle vs 0.9 \pm 0.1 in bicuculline). When quinpirole was added to bicuculline, the eEPSC amplitude decreased ($-82 \pm 21pA$), and the PPR increased (1.3 ± 0.1). Bars: A, B, and F-I, 100pA, 12.5 milliseconds; C and D, 10pA, 250 milliseconds.

Thus, the paradoxical reduction in GABA interneuron function following D2R activation was alleviated by the $GABA_AR$ antagonist.

PCE also provoked abnormal excitatory neurotransmission 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 ± 9%; p = 0.9). The addition of bicuculline to quinpirole did not change the PPR (-0.2 ± 10%), but enhanced cell excitability, as the eEPSC amplitude increased (55 ± 11%; p < 0.02 compared with vehicle or quinpirole, paired t test; see Fig 8G). Following PCE, quinpirole reduced the eEPSC amplitude (-18 ± 5%; n = 8; p = 0.02) and increased the PPR (27 ± 7%; p = 0.01). The addition of bicuculline to quinpirole increased the eEPSC amplitude (29 ± 2%; p = 0.01), whereas the PPR approached baseline (-2 ± 5%; p = 0.9 compared with vehicle; p = 0.02 compared to quinpirole, paired t test; see Fig 8H).

Discussion

PCE causes 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 (see Fig 1B). PCE promoted inappropriate dopamine filtering of corticostriatal activity, because rather than boosting stronger cortical connections while inhibiting the weak, D2Rs strengthened the weaker glutamatergic synapses (see Fig 1).¹⁵

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

Although normally promoting inhibition at corticostriatal synapses,^{15,30} D2Rs boosted corticostriatal activity

FIGURE 8: Abnormal y-aminobutyric acid (GABA) interneuron function. (A) Current clamp recordings show characteristic responses of fast spiking (FS) and (B) persistent low-threshold spiking (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 (RMPs) than saline cells. Current-voltage plots (right) show similar responses in saline- and cocaineexposed interneurons after subtraction of their RMPs (responses were measured at arrows). FS interneurons (shown in A) 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 (shown in B) 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 and a lower RMP, and a much lower input current was required to produce action potentials (see Supplementary Tables S3 and S4), with values similar to those reported previously.^{14,49} (C) Representative current clamp recordings in FS and (D) PLTS interneurons from saline-exposed (above) and cocaine-exposed mice (below) demonstrate typical responses to the GABA_A receptor antagonist bicuculline before and after bath application of the sodium channel blocker tetrodotoxin. In FS and PLTS interneurons, bicuculline depolarized saline-exposed cells (the membrane potential became more positive) to a much greater degree than cocaine-exposed cells. GABA likely produced tonic inhibition at GABA_A autoreceptors, ¹⁴ because the change in membrane potential by bicuculline in saline-exposed mice (FS, 37 ± 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-millisecond, 100pA current pulses applied every 10 seconds. 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 salineexposed mice (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 Figure S5. (G) Excitatory inputs onto green fluorescence protein (GFP)⁺ fluorescent interneurons from Lhx6-GFP transgenic mice were activated with paired pulses using cortical bipolar stimulating electrodes. The paired-pulse ratio (PPR) was similar in cells from saline-exposed mice (1.35 ± 0.11; n = 11) and cocaine-exposed mice (1.13 \pm 0.09; n = 8; p = 0.2, t test), suggesting equivalent baseline excitability. In salineexposed mice, representative traces (above) and graph show that quinpirole did not change the amplitude of the first evoked excitatory postsynaptic current (eEPSC; $-32 \pm 3pA$ in vehicle vs $-35 \pm 6pA$ following quinpirole) or the PPR (1.3 \pm 0.1 in vehicle vs 1.3 \pm 0.1 in guinpirole). When guinpirole was combined with bicuculline, the eEPSC amplitude increased (-45 \pm 6pA), but the PPR remained unchanged (1.3 \pm 0.3). The α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor antagonist NBQX abolished the eEPSC. (H) In GFP⁺ interneurons from cocaine-exposed mice, quinpirole reduced the amplitude of the first eEPSC (-62 \pm 5pA in vehicle vs -49 \pm 9pA following quinpirole), and the PPR increased (1.1 \pm 0.1 in vehicle vs 1.4 \pm 0.2 in quinpirole). Paired-pulse depression was observed in the presence of bicuculline, which prevented the change in PPR (1.1 \pm 0.2), and the eEPSC amplitude increased (-95 \pm 13pA). Similar results were found in FS and PLTS-type saline- and cocaine-exposed interneurons, with cell types discriminated based on their input resistance (not shown). Bars: A and B, 30mV, 20 milliseconds; C–F, 30mV, 2 minutes; G and H, 50pA, 12.5 milliseconds.

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 inputs,³⁹ which are also aberrantly controlled by D2Rs.

We also found that a GABAAR antagonist can prevent this paradoxical excitation of corticostriatal activity by D2Rs. Whereas GABAAR 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 GABAA autoreceptors³³ is dependent on ambient GABA supplied by striatal neurons and inputs from the external pallidum.^{33,40,41} In controls, GABAAR blockade suppressed inhibitory tonic GABA currents, indicated by interneuron depolarization. PCE hyperpolarized GABA interneurons and suppressed bicuculline-sensitive tonic GABA currents, which were reactivated by the D2R agonist. Thus, the combination of GABAAR antagonist and D2R agonist would strengthen corticostriatal inhibition at GABABRs and oppose any reduction in tonic GABA caused by the D2R agonist alone.

D2Rs located on GABA interneurons³³ modulate tonic GABA currents through PKA,³⁵ 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 downregulates 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 locomotion comparable to that of controls was achieved 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 overinhib-

ited 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 GABAergic function in the murine cortex and hippocampus following PCE.²³ Because a GABA_BR antagonist allows 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

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Authorship

I.N., J.S.L., N.M.P., G.P.S., and N.S.B. were responsible for animal production; W.W. was responsible for electrophysiology recordings; I.N. and N.S.B. were responsible for behavioral studies; J.S.L. and N.M.P. were responsible for Western blot; J.C.L. and P.E.M.P. were responsible for cyclic voltammetry; I.J.B. and N.S.B. were responsible for data analysis; N.S.B. prepared the figures and wrote the paper.

Potential Conflicts of Interest

Nothing to report.

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