

Shifts in striatal responsivity evoked by chronic stimulation of dopamine and glutamate systems

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Summary

Dopamine and glutamate are key neurotransmitters in cortico-basal ganglia loops affecting motor and cognitive function. To examine functional convergence of dopamine and glutamate neurotransmitter systems in the basal ganglia, we evaluated the long-term effects of chronic stimulation of each of these systems on striatal responses to stimulation of the other. First we exposed rats to chronic intermittent cocaine and used early-gene assays to test the responsivity of the striatum to subsequent acute motor cortex stimulation by application of the GABA_A (gamma-aminobutyric acid alpha subunit) receptor antagonist, picrotoxin. Reciprocally, we studied the effects of chronic intermittent motor cortex stimulation on the capacity for subsequent acute dopaminergic treatments to induce early-gene activation in the striatum. Prior treatment with chronic intermittent cocaine induced motor sensitization and significantly potentiated the striatal expression of Fos-family early genes in response to stimulation of the motor cortex. Contrary to this, chronic intermittent stimulation of the motor cortex down-regulated cocaine-induced gene expression

in the striatum, but enhanced striatal gene expression induced by a full D1 receptor agonist (SKF 81297) and did not change the early-gene response elicited by a D2 receptor antagonist (haloperidol). These findings suggest that repeated dopaminergic stimulation produces long-term enhancement of corticostriatal signalling from the motor cortex, amplifying cortically evoked modulation of the basal ganglia. By contrast, persistent stimulation of the motor cortex inhibits cocaine-stimulated signalling in the striatum, but not signalling mediated by individual dopamine receptor sites, suggesting that chronic cortical hyperexcitability produces long-term impairment of dopaminergic activity and compensation at the receptor level. These findings prompt a model of the basal ganglia function as being regulated by opposing homeostatic dopamine–glutamate neurotransmitter interactions. The model provides a framework for analysing the neurological alterations associated with disorders of the basal ganglia and their treatment with pharmacotherapies affecting dopamine and glutamate neurotransmitter systems.

Keywords: basal ganglia; dopamine; glutamate; motor cortex; striatum

Abbreviations: MOR1 = mu-opiate receptor

Introduction

Interactions between forebrain dopamine and glutamate systems shape the functioning of cortico-basal ganglia loops both acutely and in the long term (Carlsson and Carlsson, 1990; Schmidt, 1998; Canales and Graybiel, 2000a; Chase and Oh, 2000; Onn *et al.*, 2000; Greenamyre, 2001). Drug

treatments that alter dopamine signalling in these loops produce changes in sensorimotor and cognitive processing (Jentsch and Taylor, 1999; Graybiel *et al.*, 2000). Glutamatergic transmission has been implicated in both the neural and behavioural effects of such dopaminergic treatments (Carlsson and Carlsson, 1990; Blanchet *et al.*, 1997;

Wolf, 1998; Zigmond *et al.*, 1998). The mechanisms underlying interactions between dopamine and glutamate systems are thought to invoke multiple levels within cortico-basal ganglia circuits, and they are still poorly understood. One important clue to analysing these interactions is emerging, however, from evidence that both the glutamate and dopamine systems converge on intracellular messenger systems, including those involving dopamine and cAMP-regulated phosphoprotein, DARPP-32, and sets of genes encoding transcription factors such as the cyclic AMP responsive element binding protein and members of the Fos-Jun and NGFI-A families (Berretta *et al.*, 1992, 1997; Hyman *et al.*, 1995; Chergui *et al.*, 1997; Sgambato *et al.*, 1997; Self *et al.*, 1998). A common set of molecular mechanisms may thus operate at different points within the complex anatomical loops.

The cell and molecular biology associated with these interactions has also allowed detection of neuronal excitation by dopaminergic and glutamatergic stimulation: both evoke the expression of transcription factors that can be detected anatomically at the single-neurone level, with antibodies raised against the corresponding proteins. Through the use of such methods, it has been shown that long-lasting changes can occur in the sets of neurones activated by a range of dopaminergic treatments. For example, chronic intermittent treatment with levodopa or dopamine receptor agonist in rodent models of Parkinson's disease (Paul *et al.*, 1992; Cenci *et al.*, 1999; Saka *et al.*, 1999) and chronic exposure to psychomotor stimulants acting at dopamine receptors both produce shifts in inducibility of Fos-Jun proteins in the neocortex and the striatum (Hope *et al.*, 1994; Moratalla *et al.*, 1996; Canales and Graybiel, 2000b). These gene-based effects also depend on dopamine-glutamate interactions. The striatal expression of early-gene proteins evoked by dopaminergic stimulation requires the activation of *N*-methyl-D-aspartate receptors (Ishida *et al.*, 1996; Konradi *et al.*, 1996). Likewise, the early-gene expression elicited by acute stimulation of cerebral cortex (Fu and Beckstead, 1992; Berretta *et al.*, 1997; Parthasarathy and Graybiel, 1997; Sgambato *et al.*, 1997) is attenuated by dopamine D1-class receptor antagonists and by 6-hydroxydopamine lesions of the nigrostriatal dopamine pathway (Liste *et al.*, 1995), whereas such cortically evoked activation of striatal neurones is potentiated by D2-class dopamine receptor antagonists (Berretta *et al.*, 1999).

In the work reported here, we used early-gene expression assays to study functional interactions between dopamine and glutamate systems in animals exposed to chronic stimulation of one of the two transmitter systems. We chose chronic-intermittent cocaine treatment for its capacity to stimulate the dopamine system and to evoke correlated changes in early-gene inducibility in the striatum and increased behavioural stereotypy (Moratalla *et al.*, 1996; Canales and Graybiel, 2000b). We chose chronic intermittent epidural application of

the GABA_A (gamma-aminobutyric acid alpha subunit) antagonist, picrotoxin, to release the motor cortex locally from GABAergic inhibition and thus to activate the corticostriatal glutamatergic system (Berretta *et al.*, 1997, 1999). These paradigms allowed us to examine cross-transmitter interactions by measuring the effects of the chronic cocaine treatment on subsequent acute activation of striatal neurones by cortical stimulation, and the effects of the chronic cortical stimulation on subsequent acute activation of striatal neurones by cocaine. To ensure that the effects studied were long-term consequences of the chronic treatments, we administered the cross-transmitter challenges after a period of withdrawal. Our results suggest that chronic stimulation of either the glutamate system or the dopamine system produces long-term changes in the capacity of the other to excite the striatum, and thus to engage cortico-basal ganglia loop function.

Methods

Treatments and surgery

Sprague-Dawley rats weighing 250–300 g at the beginning of the experiments were treated according to protocols approved by the Massachusetts Institute of Technology Committee on Animal Care. Rats were handled before the experiments began and were randomly assigned to experimental groups of at least six animals. In the first set of experiments, cocaine hydrochloride (25 mg/kg; Sigma, St Louis, MO, USA) or 0.9% saline was administered intraperitoneally twice daily (at 10:00 and 17:00) for 7 consecutive days. The behaviour of the animals was studied after the morning treatment by observers blind to the experimental treatments. Following the chronic cocaine or chronic saline administration, the rats were withdrawn from treatments for 1 week and were then prepared for cortical stimulation. Each rat was anaesthetized with a combination of ketamine (75 mg/kg) and xylazine (10 mg/kg), and a burr hole 2 mm in diameter was drilled in the skull under stereotaxic guidance at antero posterior = 0.5, mediolateral = +1.5, with care taken not to damage the dura overlying primary motor cortex. A small plastic well was placed around the hole, secured with waterproof epoxy resin, dental acrylic and mounting screws, and carefully sealed (Berretta *et al.*, 1997). The following day (day 15), the well was filled with picrotoxin (200 µM; Sigma, St Louis, MO, USA) or 0.9% saline, animals were immediately placed in their home cages and their behaviour was observed by the experimenters.

In the second set of experiments, rats first underwent the surgical procedure involving implantation of the well over motor cortex, and received epidural picrotoxin (200 µM) or saline twice daily (at 10:00 and 17:00) for 7 days. For the chronic epidural treatment, picrotoxin or saline was left in the well for 1 h and then removed, and the rims and inner wall of the well were then carefully cleansed with antiseptic solution. On day 7, following the last epidural treatment, the well was

permanently sealed with dental cement, and the animals were withdrawn from the treatments. On day 15, rats received an intraperitoneal challenge with cocaine (25 mg/kg) or saline, or, in follow-up experiments, with the D1-class dopamine receptor agonist SKF 81297 (9 mg/kg; Sigma, St Louis, MO, USA) or the D2-class dopamine receptor antagonist haloperidol (1.5 mg/kg; Sigma, St Louis, MO, USA). The rats were immediately placed in their home cages for behavioural observation.

Tissue preparation and analysis

Rats were deeply anaesthetized with pentobarbital 1 h after the final drug challenge and were perfused transcardially with 4% paraformaldehyde in 0.1 M PO₄. The brains were removed, postfixed for 2 h, and stored in 20% glycerol in 0.1 M PO₄ buffer containing 0.2% solution azide. Transverse 24- μ m frozen sections were cut through the caudoputamen on a sliding microtome, and dual-antigen immunohistochemistry was carried out on series of sections with antisera raised against cFos (Ab-5, 1:40 000; Oncogene Science, Manhasset, NJ, USA, now Calbiochem, San Diego, CA, USA) and the C-terminal peptide of the mu opiate receptor (MOR1) (1:20 000; Dia Sorin, Stillwater, MN, USA) (Canales and Graybiel, 2000b). MOR1 levels are high in the striosomes and low in the matrix, and provide an excellent marker to identify these two neurochemically distinct compartments of the striatum (Fig. 2). Control sections were stained for cFos alone. The immunostained sections were analysed by light microscopy with the aid of image analysis software (Biocom, Les Ulis, France) as described in detail elsewhere (Moratalla *et al.*, 1996; Berretta *et al.*, 1997; Canales and Graybiel, 2000b). Fos-positive nuclei were counted in the MOR1-enriched striosomes and in the surrounding matrix of an ~4 mm² rectangle spanning the medial and lateral halves within the caudoputamen. Counts were carried out in one or two sections through the rostrocaudal extent of the striatum by experimenters blind to the pharmacological treatments. Fos expression data were analysed by ANOVA (analysis of variance) followed by post-hoc comparisons, where appropriate.

Behavioural observations

Motor stereotypy induced by the cocaine challenges was monitored with a modified Creese-Iversen 10-point rating scale (Canales and Graybiel, 2000b). Behaviours monitored included head bobbing and swaying, limb movements, sniffing, grooming, rearing, chewing and licking. Two scores were given, at 20 min and 50 min after the cocaine challenge, and the two were averaged to provide a single overall session score. All ratings were made by experimenters blind to the experimental treatments. Following epidural picrotoxin treatment, the presence or absence of compulsive motor tics was recorded (Berretta *et al.*, 1997). Non-parametric statistical analysis was performed on the behavioural observations.

Results

Repeated cocaine exposure enhances cortically mediated signalling in striatal neurones

In the first set of experiments we studied the gene expression patterns and motor responses evoked by cortical stimulation in cocaine-sensitized animals and drug-naïve controls. The chronic intermittent exposure to cocaine produced typical motor sensitization (Canales and Graybiel, 2000b), characterized by increasingly stereotyped motor behaviour following the repeated administration of the drug (Fig. 1). Rats receiving chronic cocaine, but not those receiving saline injections, reached values of ~5 on the stereotypy scale by day 7 of treatment. Such chronic cocaine exposure did not alter the capacity of epidural picrotoxin treatment of the motor cortex to elicit intense motor tics. The epidural picrotoxin challenge produced discrete, movements of the hindlimbs and, occasionally, also of the forelimbs in all cocaine-pretreated rats (seven out of seven), and similarly induced motor tics in each of the saline-pretreated animals (seven out of seven) (Fig. 1). These motor tics were not detectably different in the two groups. Typically, the tics appeared within 10 min of the application of picrotoxin, and lasted for the entire session.

The patterns of gene expression induced by acute picrotoxin challenge were markedly different in the cocaine-pretreated and drug-naïve animals. In the controls, acute stimulation of the motor cortex with epidural picrotoxin induced Fos expression, especially laterally in the sensorimotor sector of the caudoputamen, and there were differences in expression in the two main neurochemical compartments

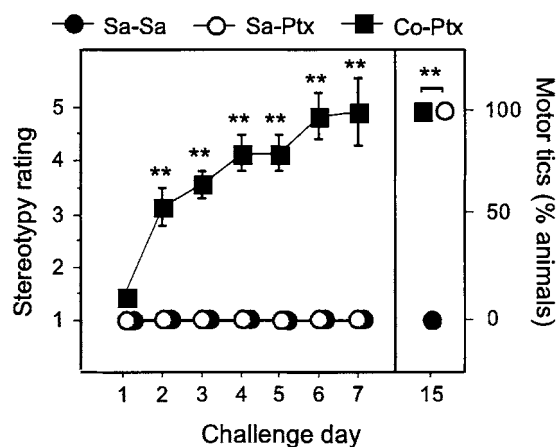


Fig. 1 Stereotypy ratings during chronic cocaine treatment and induction of motor tics after acute epidural picrotoxin challenge in cocaine-sensitized and naïve animals. Kruskal–Wallis tests showed that cocaine treatment increased stereotypy on days 2–7. Note the progressively elevated stereotypy scores after repeated cocaine challenges (ordinate to the left). Motor tics (indicated by symbols to the right of the vertical line) were evoked in all experimental animals treated with picrotoxin or saline (ordinate to the right). Sa-Sa = chronic saline–epidural saline; Sa-Ptx = chronic saline–picrotoxin; Co-Ptx = chronic cocaine–picrotoxin. ** $P < 0.01$ (Kruskal–Wallis tests).

of the striatum: expression was greater in the matrix compartment than in striosomes (Figs 2A and B, and 3). Prior chronic intermittent exposure to cocaine produced a strong potentiation of the Fos expression evoked in the medial caudoputamen by the acute motor cortex stimulation, relative to that observed in the saline-pretreated controls. This shift occurred both in the matrix compartment and in striosomes, where the cortically stimulated gene expression was enhanced by previous cocaine experience by 160 and 180%, respectively. In the lateral caudoputamen, there was only a slight increase in Fos expression after cocaine sensitization, and differences in gene expression values between naïve and cocaine-exposed animals did not reach statistical significance (Fig. 3). These results suggest that the repeated exposure to cocaine produced regional, but non-compartmental, increases in Fos expression in the caudoputamen in response to acute activation of the motor cortex, inducing a population-level increase in responsiveness of the corticostriatal mechanism that remained after 1 week of withdrawal from any drug treatment.

Persistent stimulation of motor cortex dampens subsequent activation of striatal signalling by cocaine

The fact that repeated activation of the dopamine system affected striatal responsiveness to cortical input suggested that the reverse might also be true. We therefore exposed rats to repeated epidural application of picrotoxin to the motor cortex and, after 1 week of withdrawal from such exposure, examined the striatal response to a single challenge dose of cocaine. Animals receiving the chronic intermittent picrotoxin treatment showed robust motor tics after each picrotoxin administration, and the repetition of the treatments did not reduce these motor responses as treatment progressed. All six rats exposed to the pulsatile picrotoxin treatment responded behaviourally on day 7, indicating that cortical motor output remained functional even after persistent stimulation. Following a 7-day withdrawal interval, the rats received a final challenge with cocaine (25 mg/kg). This acute cocaine administration produced modest increases of ~40% in motor stereotypy, both in the picrotoxin-pretreated and in the saline-pretreated animals (Fig. 1). The chronic epidural application of picrotoxin to the motor cortex did not appear either to facilitate or to inhibit the motor stereotypy induced by acute cocaine treatment.

The pattern of striatal Fos expression induced in the control animals by acute cocaine challenge was relatively homogeneous, both medially and laterally, and was characterized by a two- to three-fold greater expression in striosomes. These observations are consistent with those of previous studies (Moratalla *et al.*, 1996; Canales and Graybiel, 2000b). The chronic intermittent picrotoxin pretreatment produced a dramatic decrease in this early-gene response (Figs 1 and 4). The levels of striatal Fos expression elicited by acute cocaine

challenge were decreased by >60% on average in both striosomes and matrix relative to control levels in the saline-pretreated animals. Thus, the balance of activation of the two compartments was not different in the picrotoxin-pretreated and control rats, despite major differences between these groups in the overall numbers of striatal neurones activated and in the levels of activation of neurones in the individual compartments (Fig. 5).

Chronic intermittent cortical stimulation potentiates D1 receptor-mediated signalling in the striatum

Given that excessive glutamatergic stimulation is excitotoxic, one obvious possibility to account for the dampening effects of persistent stimulation of motor cortex on striatal Fos inducibility was that the treatment damaged cortical neurones, striatal neurones or both. We therefore prepared further sets of picrotoxin-treated rats and probed striatal Fos inducibility with two compounds acting at individual dopamine receptor subclasses: the selective D1-class receptor agonist, SKF 81297, and the D2-class receptor antagonist, haloperidol. Both compounds are known to induce robust gene expression in the striatum (Nguyen *et al.*, 1992; Hiroi and Graybiel, 1996; Liu and Graybiel, 1998; Berretta *et al.*, 1999).

To our surprise, we found that the same chronic intermittent cortical picrotoxin treatment that reduced cocaine-stimulated gene expression in the striatum potentiated the gene expression response evoked by the D1 agonist SKF 81297 (Figs 2 and 6). In rats exposed to chronic intermittent stimulation of motor cortex and withdrawal, acute SKF 81297 administration elevated levels of Fos expression in both striosomes (by 150%) and matrix (by 260%) in the medial caudoputamen relative to values for rats pretreated with epidural saline (Fig. 6). Thus, repeated cortical stimulation amplified D1 receptor-mediated striatal responses in this striatal sector. We could not detect any effect of the picrotoxin-pretreatment in the lateral caudoputamen, in which cocaine-induced Fos expression had already been reduced by the prior chronic cortical stimulation.

Acute challenge with the D2 antagonist haloperidol potentially increased gene expression in the lateral caudoputamen in the second group of rats given repeated application of picrotoxin and withdrawal, and this striatal gene response was not detectably different from that induced by acute haloperidol administration in the saline-pretreated controls (Fig. 7). Thus, neither challenge with the D2-antagonist probe known to activate Fos in enkephalinergic striatal neurones (Berretta *et al.*, 1992), nor activation of D1-class dopamine receptors, which induces Fos in dynorphin-containing striatal neurones (Steiner and Gerfen, 1995), suggested impairment in the capacity of striatal neurones to express Fos following chronic picrotoxin-induced cortical stimulation.

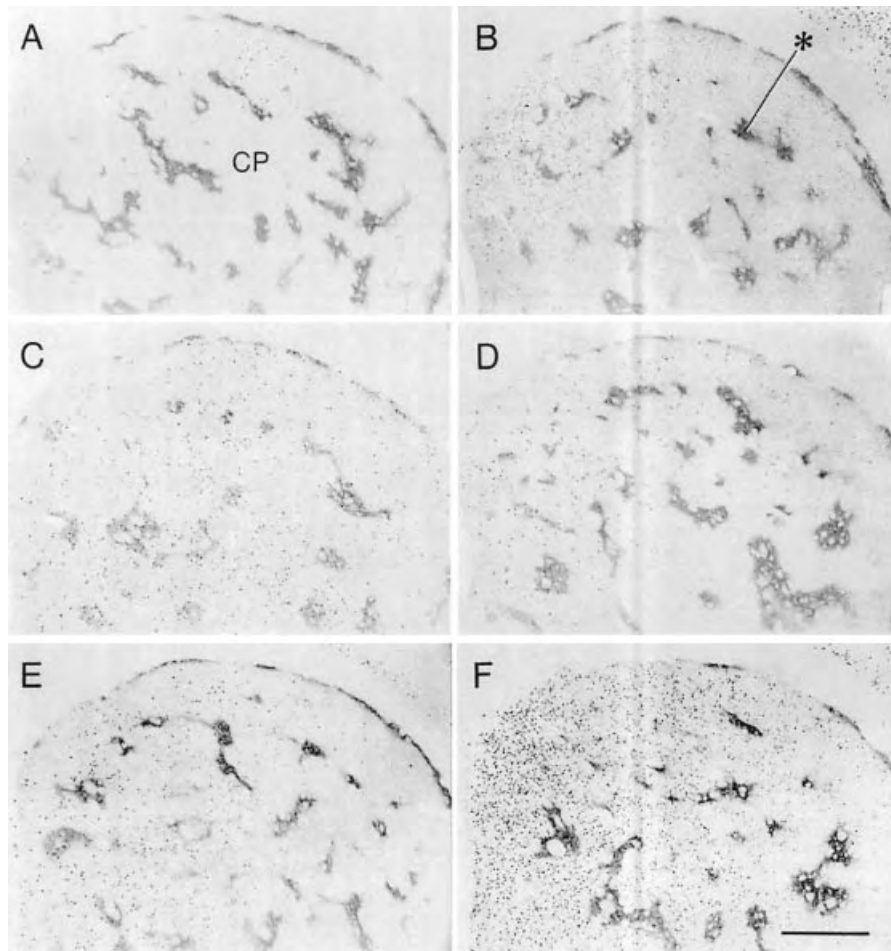


Fig. 2 Reciprocal influences of chronic intermittent cortical stimulation and dopamine agonist treatments on Fos expression in the striatum. Each panel illustrates a section through the caudoputamen, double-stained to demonstrate MOR1-positive striosomes [see asterisk in (B)] and Fos-positive nuclei induced by challenge following the chronic intermittent treatment and withdrawal. For each pair of panels (A–B, C–D, E–F), the left side (A, C, E) illustrates a control experiment in which chronic intermittent saline treatment and withdrawal preceded the challenge. (A, B) Fos expression induced by acute challenge with epidural picrotoxin application to disinhibit motor cortex after chronic intermittent saline treatment and withdrawal (A), and after chronic intermittent cocaine treatment (B). Note up-regulation of Fos expression in (B). (C, D) Fos expression induced by acute challenge with cocaine following chronic intermittent treatment with saline (C) and epidural picrotoxin (D). Note down-regulation of Fos expression in (D). (E, F) Fos expression induced by acute challenge with the D1 agonist, SKF 81297, following chronic intermittent treatment with saline (E) and epidural picrotoxin (F). Note up-regulation of Fos expression in (F). CP = caudoputamen. Scale bar = 0.5 mm.

Discussion

Selective targeting of neuronal dopamine and glutamate neurotransmitter systems represents a major strategy for treating the motor and cognitive signs of cortico-basal ganglia dysfunction. In the experiments reported here, we developed animal models to test the effects of stimulating each of these neurotransmitter systems with chronic intermittent schedules of drug administration. Our results with Fos assays suggest that interactions between these dopamine and glutamate signalling systems lead to changes in the responsivity of each system after stimulation of the other. First, we demonstrate that long-lasting alterations in cortico-basal ganglia signalling can occur as a result of chronic intermittent stimulation of dopamine receptors. These alterations were detected in the

striatum as an amplified response to cortical stimulation in animals pre-exposed to a regimen of chronic intermittent cocaine administration. Secondly, we demonstrate that long-lasting alterations in dopaminergic responsivity occur in the striatum as a result of chronic intermittent stimulation of the motor cortex, which gives rise to glutamatergic corticostriatal inputs. These changes included a decrease in responsivity to strong dopaminergic challenge, but apparently involved multiple compensations. Together, these findings suggest the presence of homeostatic dopamine–glutamate interactions in the control of cortico-basal ganglia output and suggest that cross-transmitter effects should be a focus in assessments of therapies designed to target each of these neurotransmitter systems individually.

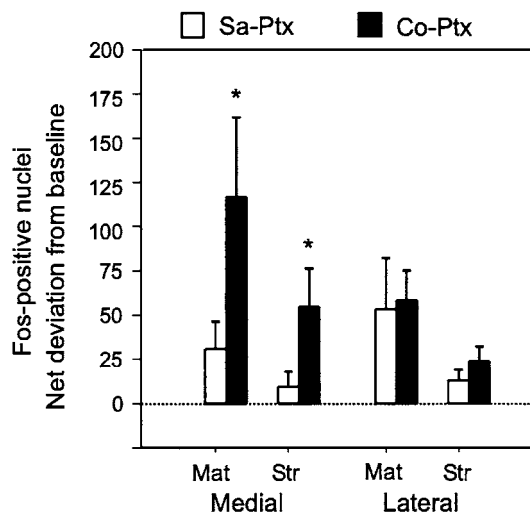


Fig. 3 Fos-positive cell counts in the caudoputamen after acute epidural application of picrotoxin in cocaine-sensitized and naïve animals. Values are expressed as deviations from baseline (chronic intraperitoneal saline followed by acute epidural saline). Exposure to chronic cocaine treatment potentiated cortically stimulated Fos expression in the medial sector of the caudoputamen. ANOVA indicated a significant effect of pretreatment [$F(2,16) = 5.104, P = 0.0193$]. Sa-Ptx = chronic saline-epidural picrotoxin; Co-Ptx = chronic cocaine-epidural picrotoxin; Mat = matrix; Str = striosome. * $P < 0.05$ (Newman-Keuls tests).

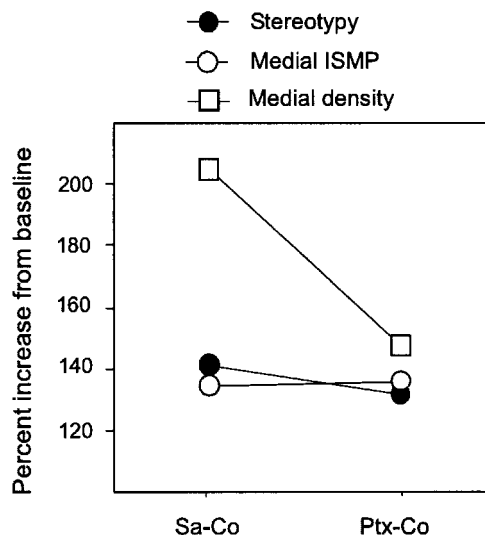


Fig. 5 Mean percentage of increase relative to baseline values (chronic epidural saline treatment followed by acute intraperitoneal saline) on measures of stereotypy, index of striosome-to-matrix predominance (ISMP) and overall density in the medial sector of the caudoputamen following acute cocaine treatment. Note that the decreased expression of Fos in the chronic picrotoxin-cocaine group is unrelated to stereotypy levels induced by acute cocaine challenge. However, levels of stereotypy match ISMP values estimating the relative activation of striatal compartments. Sa-Co = chronic epidural saline-cocaine; Ptx-Co = chronic epidural picrotoxin-cocaine; ISMP = index of striosome-to-matrix predominance.

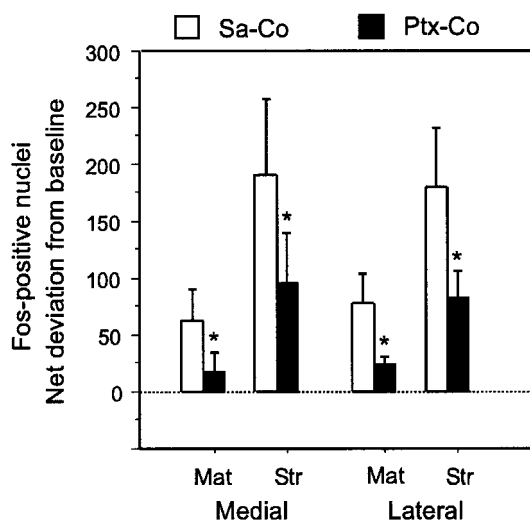


Fig. 4 Counts of Fos-positive nuclei in the caudoputamen following cocaine treatment in naïve and cortically stimulated animals, expressed as deviations from baseline (chronic epidural saline treatment followed by acute intraperitoneal saline). Chronic epidural picrotoxin produced a widespread decrease in Fos expression evoked by cocaine challenge in the matrix and striosomes, within medial and lateral sectors of the caudoputamen. ANOVA indicated a significant effect of pretreatment [$F(2,15) = 5.063, P = 0.021$], and no interaction with either the region (medial, lateral) or compartment (matrix, striosome) factors. Sa-Co = chronic epidural saline-cocaine; Ptx-Co = chronic epidural picrotoxin-cocaine; Mat = matrix; Str = striosome. * $P < 0.05$ (Newman-Keuls tests).

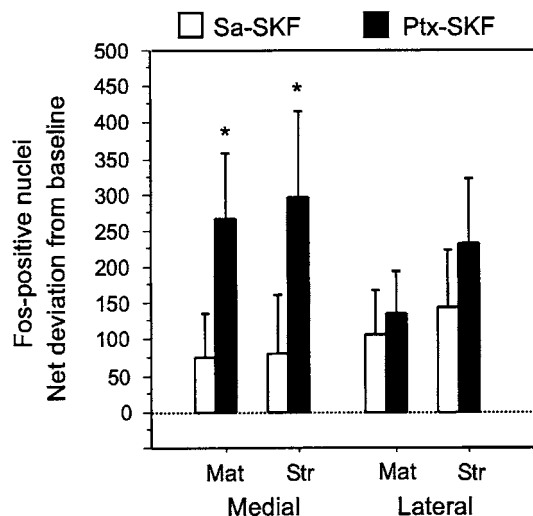


Fig. 6 Induction of Fos protein in the caudoputamen by SKF 81297 (9 mg/kg) following exposure to repeated motor cortex stimulation with picrotoxin or saline, expressed as net deviations from baseline (chronic epidural saline treatment followed by acute intraperitoneal saline). ANOVA showed no overall effect of pretreatment [$F(2,14) = 2.760, P = 0.098$] but a significant pretreatment/region interaction [$F(2,14) = 4.100, P = 0.039$], indicating a region-dependent potentiated effect of SKF 81297 after chronic cortical stimulation treatment. Sa-SKF = chronic epidural saline-SKF 81297; Ptx-SKF = chronic epidural picrotoxin-SKF 81297; Mat = matrix; Str = striosome. * $P < 0.05$ (Newman-Keuls tests).

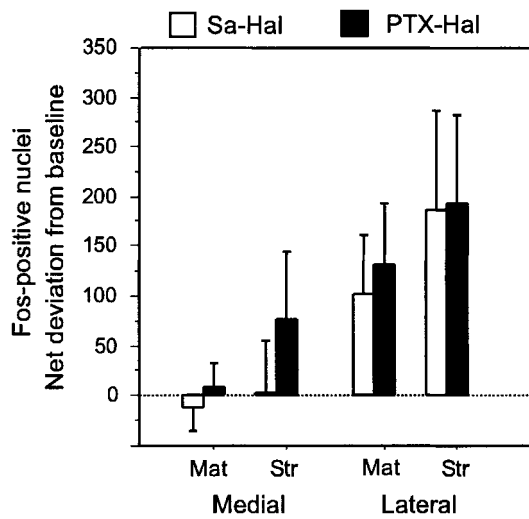


Fig. 7 Counts of Fos-positive nuclei in the matrix and striosomes of the caudoputamen following challenge with haloperidol (1.5 mg/kg) in animals exposed previously to epidural picrotoxin or saline, expressed as net deviations from baseline (chronic epidural saline treatment followed by acute intraperitoneal saline). ANOVA indicated no significant effects of pretreatment or of pretreatment/region interaction, indicating that chronic picrotoxin stimulation did not affect haloperidol-induced striatal gene expression. Sa-Hal = chronic epidural saline–haloperidol; Ptx-Hal = chronic epidural picrotoxin–haloperidol; Mat = matrix; Str = striosome.

Chronic-intermittent exposure to cocaine facilitates corticostriatal signalling from motor cortex in striatal neurones

Changes in inducibility and/or expression patterns of genes of both early-response and late-response classes occur in the rodent striatum when chronic intermittent cocaine or amphetamine treatments are followed, even after weeks of withdrawal, by another exposure to the same dopaminergic drug (Hope *et al.*, 1994; Moratalla *et al.*, 1996; Canales and Graybiel, 2000b; Vanderschuren and Kalivas, 2000). Comparable changes occur following chronic intermittent levodopa or dopamine receptor agonist treatments in dopamine-depleted rats (Paul *et al.*, 1992; Andersson *et al.*, 1999; Cenci *et al.*, 1999; Saka *et al.*, 1999; Canales and Graybiel, 2000a). Because many of the signalling systems in striatal neurones are convergence points for dopamine and glutamate signalling, we hypothesized that we might find alterations in responses to stimulation of corticostriatal glutamatergic inputs to the striatum after stimulation of the dopamine system. Our findings supported this view. After chronic intermittent cocaine treatment and withdrawal, there were increases exceeding 250% in the numbers of striatal neurones induced to express Fos, a transcription factor of the AP-1 complex, when we then acutely stimulated the motor cortex.

At first glance, it seems surprising that this amplified response was mainly in the medial caudoputamen rather than

in the lateral sensorimotor sector, to which the motor cortex projects. However, stimulation of the motor cortex not only focally activates projection neurones in the sensorimotor sector, but also activates a much wider striatal region, as judged by the broad distribution of Fos-positive striatal interneurones, specifically those expressing parvalbumin. Changes in network activity of these or other interneurones could lead to the larger field of activation we observed with focal motor cortex stimulation.

A number of molecular events affecting glutamate receptor function may have contributed to the amplified corticostriatal responsivity that occurred as a result of the chronic intermittent dopaminergic stimulation. Previous reports have demonstrated changes in the density and binding properties of ionotropic and metabotropic glutamate receptors after chronic cocaine exposure and the signal transduction pathways linked to them. Chronic intermittent cocaine treatment and withdrawal increases striatal mGluR5 mRNA levels (Ghasemzadeh *et al.*, 1999), and activation of such receptors positively modulates ionotropic glutamatergic transmission (Cartmell and Schoepp, 2000). Increased striatal expression of the NR2B subunit of the *N*-methyl-D-aspartate receptor has also been detected immunocytochemically following repeated cocaine exposure and withdrawal (Loftis and Janowsky, 2000). Moreover, phosphorylation of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptor subunit GluR1 is increased in the striatum by the psychomotor stimulants, cocaine and amphetamine (Snyder *et al.*, 2000).

In addition to glutamate receptor proliferation and sensitization, an abnormal facilitation or impaired inhibition of glutamate release and/or uptake could also contribute to potentiated corticostriatal signalling in striatal neurones after chronic cocaine exposure. Cocaine sensitization has been consistently associated with enhanced glutamatergic transmission at the level of midbrain dopamine neurones (Wolf, 1998; Vanderschuren and Kalivas, 2000). Furthermore, in chronically cocaine-treated animals, glutamate release is increased in the nucleus accumbens by cocaine challenge relative to levels in saline-treated controls (Reid and Berger, 1996).

Finally, changes in dopamine receptor function and signalling resulting from the chronic cocaine exposure could themselves alter the efficacy of corticostriatal transmission by virtue of pre- or post-synaptic interactions. Blockade of dopamine D2-class receptors enhances the gene expression in the striatum that is stimulated by cortical stimulation in otherwise normal rats (Berretta *et al.*, 1999). Mutant mice lacking D2 dopamine receptors have increased glutamatergic transmission in the striatum (Cepeda *et al.*, 2001). Moreover, in D2-receptor deficient mice, tetanic stimulation of corticostriatal fibres in slice preparations produces long-term potentiation in the striatum instead of long-term depression (Calabresi *et al.*, 1997). Thus, impaired D2 dopamine receptor function consequent to the chronic intermittent cocaine treatment could lead to abnormal

processing of sensorimotor signals through amplified cortical-basal ganglia signalling.

Whatever molecular mechanisms underlie the amplified corticostriatal signalling that occurs with cocaine sensitization, our findings suggest that such enhanced corticostriatal responsiveness could contribute to the hyperkinetic syndromes typical of psychomotor stimulant abuse. Our findings also raise the possibility that activity in other cortical areas implicated in the processing of drug-associated cues and the triggering of drug-seeking behaviours could also undergo amplification through altered cortical-basal ganglia loop function. We did not test systematically for such behavioural changes, but we did record the presence or absence of motor tics following stimulation of the motor cortex as a positive control for the effects of epidural picrotoxin application. We found no clear-cut differences in this behavioural measure between the cocaine-exposed and cocaine-naïve animals, presumably because the cortical distribution by picrotoxin directly affected corticospinal activity. Experiments involving other behavioural measures are nevertheless in keeping with a facilitatory effect of chronic cocaine exposure on glutamate receptor-mediated motor effects. For example, rodents exposed to cocaine showed more intense tail twitches, 'wet dog' shaking and convulsions in response to systemic glutamate receptor agonist challenge (Itzhak, 1995; Huber *et al.*, 2001).

Chronic intermittent stimulation of the motor cortex impairs cocaine-induced responses in striatal neurones, but preserves or potentiates responses of dopamine receptor subclasses

We show here that chronic intermittent activation of corticostriatal pathways by recurring picrotoxin treatment down-regulates the striatal Fos response to a subsequent acute challenge with cocaine. Unlike chronic intermittent cocaine exposure, the repeated bouts of motor cortex hyperexcitability decreased cocaine-induced neuronal activation in both striosomes and matrix of the striatum, without affecting the relative activity of the two striatal compartments (measured as the Fos activation ratio in the two compartments). This finding is consistent with our behavioural observations in that the chronic intermittent cortical stimulation also did not alter the degree or type of motor stereotypy elicited by subsequent cocaine challenge. The enhanced motor stereotypy induced by cocaine and a range of other dopaminergic treatments is correlated with differentially enhanced Fos expression in striosomes relative to that in the matrix (Canales and Graybiel, 2000b). Thus, our chronic intermittent motor cortex stimulation schedule does not fully reproduce the neural phenotype of cocaine sensitization, which features increases in the relative activation of the striosomal compartment.

The same protocol for chronic intermittent stimulation of motor cortex produced marked potentiation of striatal early-gene activation in response to D1 agonist challenge with SKF

81297, whereas challenge with haloperidol, a potent D2-class dopamine receptor antagonist, produced similar levels of striatal Fos expression in naïve and cortically stimulated animals. Population differences in glutamate receptor expression and differential induction of plasticity in striatal neurones could, in part, account for such pharmacologically specific effects. The induction of either long-term potentiation or long-term depression in striatal neurones after stimulation of corticostriatal fibres partly depends on the interaction between D1- and D2-class dopamine receptors (Calabresi *et al.*, 1999). Our findings are limited to the specific drugs we used to probe the activation of D1- and D2-class dopamine receptors, and therefore leave open the question of how cortical hyperexcitability might affect the function of different isoforms and subtypes of dopamine receptors. One interesting possibility is that the differential effects of cocaine, SKF 81297 and haloperidol following repeated disinhibitory release of corticostriatal transmission could reflect differential interactions in subsets of striatal neurones undergoing long-term potentiation or long-term depression after the chronic intermittent activation of the motor cortex.

The differences in Fos response that we found with cocaine, SKF 81297 and haloperidol challenges suggest that the decreased response to cocaine challenge after repeated motor cortex stimulation was not linked to either cell death or down-regulation of dopamine receptors, or abnormalities in their associated molecular transduction pathways. Our observations with these three different drug challenges would be consistent with reduced dopamine release, together with enhanced dopamine receptor responsiveness, following chronic intermittent cortical stimulation and withdrawal. The long-term effects of recurrent motor cortical excitation on the integrity of striatal dopaminergic synapses, the availability of dopamine transporters and the release of dopamine have not, however, been studied systematically.

A model of homeostatic dopamine–glutamate interactions

Our findings suggest that repeated exposure to cocaine, an indirect dopamine receptor agonist, disrupts signalling in the basal ganglia by abnormally facilitating signal transmission in corticostriatal pathways. Cocaine abuse in humans has been linked to lasting decreases in dopamine release and reduced striatal D2-class dopamine receptor binding (Kuhar and Pilotte, 1996; Volkow *et al.*, 1999). In rodents, blockade of dopamine D2-class receptors potentiates stimulated gene expression in the striatum evoked by motor cortex stimulation (Berretta *et al.*, 1999). Dysfunction of dopamine D2-class receptors could therefore be key to the amplified corticostriatal responsiveness that we observed in animals exposed to chronic cocaine.

We used recurrent stimulation of the motor cortex with picrotoxin, which blocks local inhibitory circuits in the

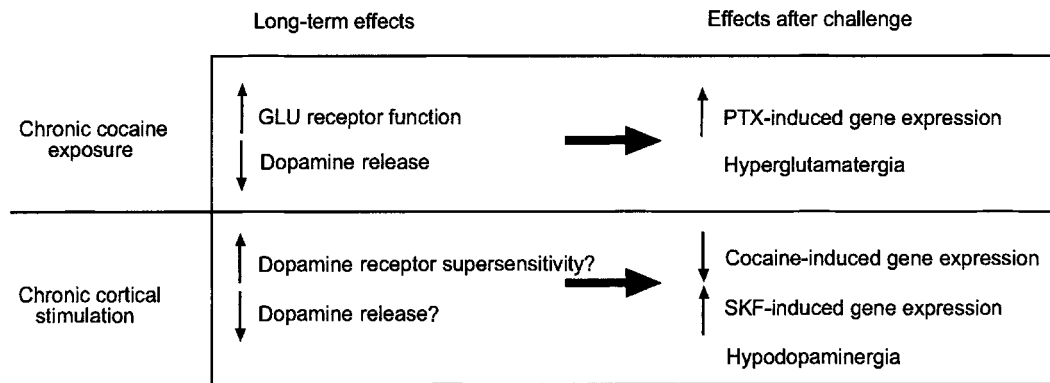


Fig. 8 Model of homeostatic dopamine–glutamate interactions, by which reciprocal effects on transmission regulate the long-term effects of chronic dopaminergic stimulation and chronic cortical hyperactivation.

cortex, to model some aspects of motor cortex hyperexcitability syndromes in humans. Motor cortex hyperactivation has been reported in dyskinetic parkinsonian patients (Rascol *et al.*, 1998), and in patients with obsessive-compulsive disorder (Greenberg *et al.*, 2000) and Tourette's syndrome (Ziemann *et al.*, 1997). We found that repeated stimulation of the motor cortex inhibited cocaine-stimulated striatal gene expression, yet potentiated (for D1-class agonists) or preserved (for D2-class agonists) the striatal gene expression evoked by drugs acting at individual dopamine receptor subclasses. Our results with repeated picrotoxin-induced stimulation are thus consistent with the possibility that the over-activation of motor cortex led to decreased dopamine release (hence a reduced effect of subsequent treatment with cocaine, which blocks the dopamine transporter) and a subsequent compensatory up-regulation of D1-class dopamine receptors (hence a heightened effect of subsequent treatment with SKF 81297). Interestingly, our results also suggest that D1-class and D2-class dopamine receptor function may be differently affected by cortical hyperexcitability. D1-class receptor function, which is associated with direct pathway activation, appears augmented, but synergistic D1-D2 function, associated with coactivation of the direct and indirect pathways, appears depressed.

Our findings with these two paradigms of chronic drug exposure adhere to a simple homeostatic model of dopamine–glutamate transmitter interactions in cortico-basal ganglia circuits (Fig. 8). Our results with chronic intermittent psychomotor stimulant exposure suggest that long-term dopaminergic treatment and withdrawal would lead to a state of hyperglutameria, abnormally potentiating cortical signalling in the basal ganglia. In turn, our results with the paradigm of chronic cortical stimulation suggest that chronic cortical hyperexcitability would induce a state of hypodopaminergia, impairing dopamine function and modulation of striatal output by dopamine receptors. These effects represent assumptions of a single functional scheme whereby, in actively interacting cortico-basal ganglia systems, dopamine

and glutamate dynamically shape information flow through opposing homeostatic mechanisms. This model of systems-level homeostasis suggests that in chronic disorders of the basal ganglia, therapeutic interventions should be critically aimed at restoring balanced activity of dopamine and glutamate neurotransmission at corticostriatal synapses.

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