

# A measure of striatal function predicts motor stereotypy

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**To identify basal ganglia circuit dysfunctions that might produce repetitive behaviors known as motor stereotypies, we applied psychomotor stimulants and a direct dopamine receptor agonist to induce different levels of stereotypy in rats. We then used a gene induction assay to measure the functional activation of neurons in the neurochemically distinct compartments of the striatum, the striosomes and the extrastriosomal matrix. The amount by which activation in the striosomes exceeded activation in the matrix predicted the degree of motor stereotypy induced by the drug treatments. These results suggest that imbalance between compartmentally organized basal ganglia circuits may represent a neural correlate of motor stereotypy.**

Stereotypy is a cardinal feature of a broad range of neurologic and neuropsychiatric disorders and is a major component of the behavioral syndrome induced by psychomotor stimulant drugs<sup>1–5</sup>. The repetitive behaviors that typify stereotypy range from repetitions of single or multiple movements (motor stereotypies) to repetitive, inflexible patterns of attention, emotion, planning and cognition typifying some clinical disorders in humans. Experimental evidence suggests that the basal ganglia are central to the expression of stereotypies. Motor stereotypies can be induced by dopaminergic stimulation of the striatum and can be abolished by intrastriatal blockade of dopaminergic transmission<sup>6–10</sup>. Clinical evidence also implicates basal ganglia dysfunction in complex behavioral and cognitive stereotypies<sup>1–3</sup>. Abnormal functioning of loop circuits interconnecting the basal ganglia and frontal cortex is thought to be critical for these effects<sup>11–13</sup>. Very little is known yet, however, about the mechanisms by which activity in basal ganglia circuits might produce stereotypy.

To approach this issue from a new viewpoint, we used two well established rodent models to induce motor stereotypy in rats and correlated the levels of stereotypy shown by the animals with the degree of activation of striatal neurons as determined by early response gene assay<sup>14–16</sup>. We reasoned that, if stereotypy requires activation of striatal neurons, we should find a tight correlation between the numbers of activated striatal neurons and the levels of stereotypy shown by the same animals. We based the first model on the observation that psychomotor stimulants such as amphetamine and cocaine, which stimulate dopamine D1-class and D2-class receptors indirectly by releasing dopamine (amphetamine) or blocking dopamine reuptake (cocaine), induce stereotypy, and do so at increasing levels when administered repeatedly<sup>17,18</sup>. We applied each of these psychomotor stimulants acutely and chronically. We based the second behavioral model on the finding that combined stimulation of D1-class and D2-class dopamine receptors produces stereotypy dose dependently in rats. We applied the D1/D2 dopamine receptor agonist apomorphine over a range of doses. We took advantage of the findings that each of these stereotypy-inducing treatments acti-

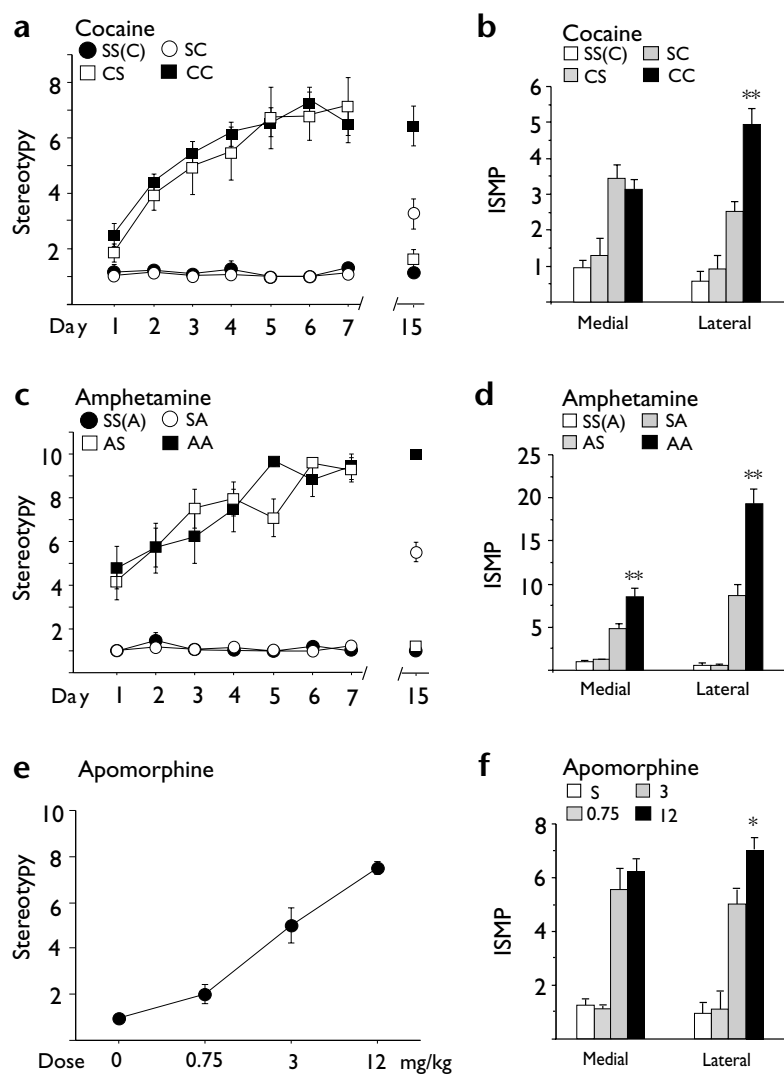
vates genes of the *fos-jun* family in striatal neurons<sup>14,19,20</sup> and that the induction can be detected immunohistochemically with a single-cell level of resolution<sup>14,16,21</sup>. This method allowed us to identify the phenotypes of the activated neurons and also to differentiate the levels of neuronal response in the two neurochemically distinguished compartments of the striatum, the striosomes and the extrastriosomal matrix<sup>14,21</sup>. Our findings demonstrate that for all of the treatment schedules, the differential activation of neurons in striosomes proved to be a remarkably accurate predictor of stereotypy.

## RESULTS

For each drug treatment, we estimated the levels of motor stereotypy by means of a modified Creese-Iversen 10-point rating scale<sup>8</sup>. To examine the neuronal changes induced by the different treatments, we used histochemical measures to detect Fos, Fra and Jun B proteins. We quantified Fos-positive striatal neurons identified by their chemical phenotype and by their compartmental location in either striosomes or matrix<sup>14</sup>. To estimate the relative gene induction in the two striatal compartments, we calculated an index of striosome to matrix predominance (ISMP), where the ISMP is the ratio between the density of Fos-positive neurons in the striosomes and the density of Fos-positive neurons in the matrix.

The motor stereotypies elicited by acute cocaine treatment (25 mg per kg; Fig. 1a) were weak, as reported previously<sup>22</sup>, and principally involved locomotor activity and rearing behavior. However, following chronic treatment (25 mg per kg, twice daily for 7 days), the same dose of cocaine induced progressively more intense stereotypies with average rating scores of ~7. This effect persisted through the 1-week withdrawal period (Fig. 1a), after which cocaine elicited perseverative movements of the head (head bobbing) and a stereotyped pattern of rearing and sniffing behavior.

In the striatum of the chronically treated rats, the final cocaine challenge induced much less Fos, Fra and JunB staining in the lateral matrix compartment than seen after acute cocaine, as well



**Fig. 1.** Cocaine, amphetamine and apomorphine treatments induce motor stereotypy and increased relative striosomal activation (ISMP ratios) in the caudoputamen. Cocaine (a) and amphetamine (c) elicited increased motor stereotypy following chronic intermittent administration (for cocaine, Kruskal-Wallis  $H = 21.163$ ,  $p < 0.0001$ ; for amphetamine, Kruskal-Wallis  $H = 21.333$ ,  $p < 0.0001$ ). Stereotypy in response to challenge persisted after withdrawal from chronic drug treatment. Apomorphine (e) increased stereotypy dose dependently (Kruskal-Wallis  $H = 21.729$ ,  $p < 0.0001$ ). For cocaine, the ANOVA for ISMP values showed a significant drug effect ( $F_{3,29} = 40.601$ ,  $p < 0.0001$ ). *Post-hoc* tests showed a significant increase in ISMP values in the lateral caudoputamen ( $q = 7.75$ ,  $p < 0.01$ ), but not in the medial caudoputamen (b). For amphetamine, the ANOVA for ISMP values showed a significant drug effect ( $F_{3,27} = 100.080$ ,  $p < 0.0001$ ). There were significant differences between acute and chronic treatments both medially ( $q = 4.85$ ,  $p < 0.01$ ) and laterally ( $q = 13.51$ ,  $p < 0.01$ ; d). For apomorphine, the ANOVA for ISMP values showed a significant drug effect ( $F_{3,22} = 52.770$ ,  $p < 0.0001$ ). The high dose of apomorphine (12 mg per kg) significantly increased ISMP values in the lateral caudoputamen compared to the middle dose (3 mg per kg;  $q = 5.04$ ,  $p < 0.05$ ; f). S, saline; SS(A), chronic saline-saline control for amphetamine cases; SS(C), chronic saline-saline control for cocaine cases; SC, chronic saline-cocaine; CS, chronic cocaine-saline; CC, chronic cocaine-cocaine; SA, chronic saline-amphetamine; AS, chronic amphetamine-saline; AA, chronic amphetamine-amphetamine. \* $p < 0.05$ , \*\* $p < 0.01$ .

as slightly more Fos staining in the striosomes (Fig. 2a and b). These changes resulted in significantly increased ISMP values (Fig. 1b). When we calculated the correlations between the stereotypy values and the indices of neural activation in the striatum, we found that the best predictor of the stereotypy was the ISMP value in the rostromedial striatum ( $r_s = 0.82$ ;  $p < 0.0002$ ), with ISMP values in other striatal regions showing lower correlations with stereotypy scores (Fig. 1b). In the same rostromedial sector, the correlation for overall density of induction was  $r_s = 0.53$  and for density in the matrix,  $r_s = 0.49$ . Even the correlations for striosomal induction in the same sector were lower ( $r_s = 0.68$ ) than the correlations for the ISMP values (Fig. 4a–d). These findings suggest that levels of motor stereotypy induced by acute and chronic cocaine treatment could be confidently predicted from the gene-based ISMP values.

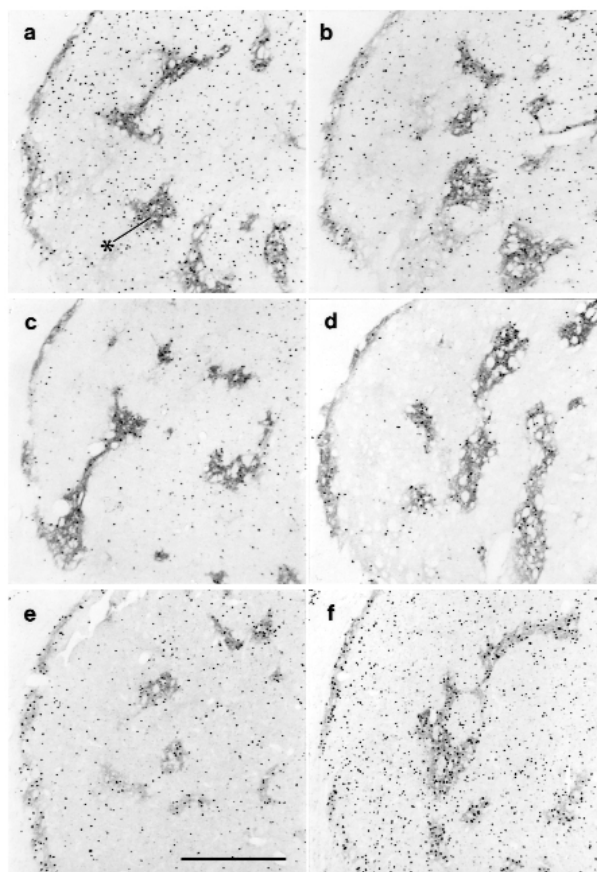
To test the generality of these observations, we carried out a second series of experiments with amphetamine, a psychomotor stimulant that triggers dopamine release<sup>5</sup> and evokes levels and patterns of motor stereotypy<sup>22</sup> and striatal early-gene response that differ from those induced by cocaine<sup>14,19–21</sup>. Acute amphetamine treatment produces a striosome-enhanced pattern of early gene expression in the striatum<sup>14</sup>, one that should be correlated with the expression of motor stereotypy. A single dose of amphetamine (5 mg per kg) induced persistent head-down sniffing, rear-

ing and bursts of prominent locomotion at stereotypy scoring levels of ~5 (Fig. 1c). After chronic exposure and withdrawal, the same dose of amphetamine induced intense, repetitive and spatially confined motor stereotypies, mainly highly repetitive up-and-down head movements, with all animals scoring the maximum stereotypy rating of 10 (Fig. 1c).

We found equally striking changes in the patterns of early gene expression in the striatum. In the rats treated chronically with amphetamine, there was a severe down-regulation of immunodetectable Fos, Fra and JunB in the matrix, leaving nearly all of the expression in the neurons of striosomes (Figs. 2c and d and 3) and significantly elevating ISMP values (Fig. 1d). This finding suggests that the two striatal compartments have different vulnerabilities to the drug treatment across a large part of the sensorimotor and associative striatum.

The critical issue in these experiments was whether the stereotypy levels elicited by the acute and chronic amphetamine treatments showed a selectively high correlation with the ISMP values for early gene induction in the caudoputamen. We found that they did. The correlations were highest for the rostromedial caudoputamen, followed by mid-level lateral caudoputamen. Values for the medial caudoputamen at rostral and mid-levels were poorer predictors of stereotypy. For the rostromedial caudoputamen,  $r_s$  was 0.92 ( $p < 0.0001$ ), indicating that amphetamine-induced motor stereotypy could be predicted with less than 8% error. By contrast, none of the regional measures of total density of responding neurons showed strong correlations with the stereotypy scores, even in the most affected rostromedial stri-

**Fig. 2.** Fos expression in the striosomal compartment is increased relative to expression in the matrix by chronic psychomotor stimulant treatments and by increasing doses of apomorphine. Photomicrographs of sections double stained to show Fos-positive nuclei (black dots) and striosomes (gray patchy zones; example indicated by asterisk in **a**) from the striatum of rats given acute (**a**) or chronic (**b**) amphetamine, acute (**c**) or chronic (**d**) cocaine, or low- (3 mg per kg; **e**) or high-dose (12 mg per kg; **f**) apomorphine. Scale bar, 500  $\mu$ m.



tum ( $r_s = 0.47$ ). Early gene induction in the matrix also failed to predict the stereotypies (for rostralateral matrix,  $r_s = 0.33$ ). The densities of responding neurons in the striosomes themselves had far less predictive power ( $r_s = 0.74$  for striosomes in the rostralateral striatum) than the ISMP values for the same sector (Fig. 4a–d). These results suggest that a relatively enhanced pattern of striosomal activation could be a neural abnormality accompanying the expression of amphetamine-induced motor stereotypy.

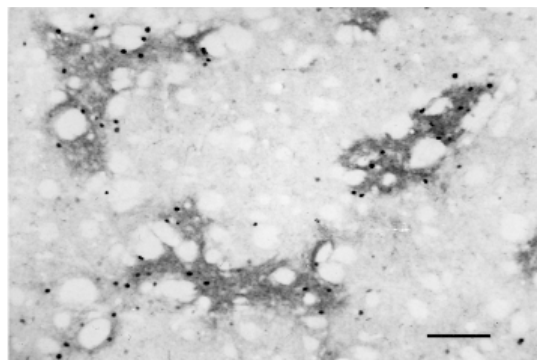
Cocaine and amphetamine enhance dopaminergic transmission, but their effects on behavior and gene expression could depend, at least in part, on the activity of the serotonergic system<sup>23,24</sup>. To test the hypothesis that dopamine receptor stimulation could induce parallel increases in stereotypy and striosome-enhanced patterns of gene expression, we induced motor stereotypy with the dopamine D1/D2 receptor agonist, apomorphine. Acute apomorphine treatment induced stereotypy in a dose-dependent fashion (Fig. 1e). The motor responses evoked were qualitatively different from those elicited by the cocaine or amphetamine treatments. The two higher doses of apomorphine (3 and 12 mg per kg) produced directed oral stereotypies, most notably licking and nibbling behaviors, that were not induced by the cocaine or amphetamine injections. In the striatum, chronic psychomotor stimulant treatment down-regulated gene inducibility in the matrix compartment, whereas apomorphine produced, as we expected, a dose-dependent increase in overall striatal early gene induction, including an increase in induction in the matrix (Fig. 2e and f). Even so, apomorphine produced a greater dose-dependent potentiation of gene expression in the striosomes than in the matrix, thereby increasing ISMP values in the lateral caudoputamen (Fig. 1f). The expression of a striosome-predominant pattern of activation could therefore occur in the presence of upward as well as downward shifts in activation of the matrix compartment.

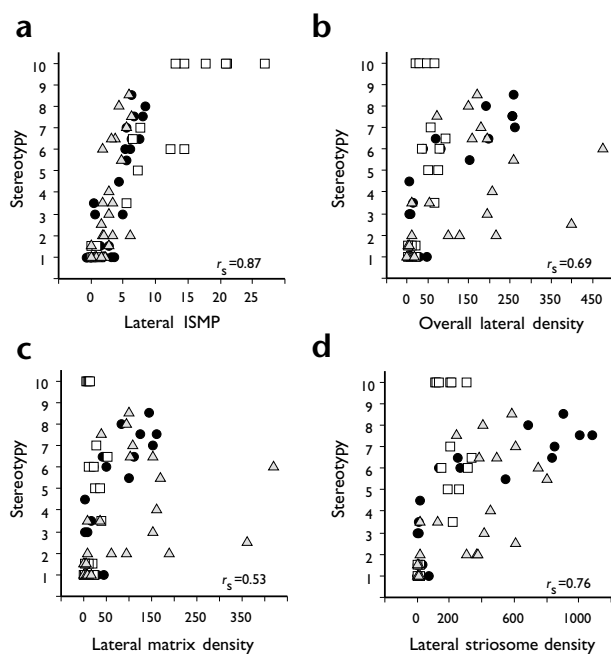
For the apomorphine treatments, as for the cocaine and amphetamine treatments, the early gene activation patterns for the rostralateral striatum were those most tightly associated with the stereotypy scores. Because the increases in density values for gene expression were dose dependent, we found good correlations between the stereotypy scores and the estimates of overall density, particularly in the rostralateral striatum ( $r_s = 0.77$ ). Correlations between the stereotypy scores and the density estimates in individual compartments in the same sector were also high ( $r_s = 0.72$  for the matrix and  $r_s = 0.83$  for the striosomes). However, as found for the two psychomotor stimulants, the best predictor of apomorphine-induced motor stereotypy was the ISMP

value ( $r_s = 0.88$ ;  $p < 0.0001$ ; Fig. 4a–d), reflecting apomorphine's production of greater dose-dependent gene induction in the striosomes than in the matrix, despite increases in gene induction in both compartments. These results suggest a critical interdependence of the two striatal compartments in the control of dopamine receptor-mediated motor stereotypy.

Compared with other measures of striatal activation, the ISMP value was the most sensitive estimate of drug-induced stereotypy for all three experiments. The only outliers were some of the data for the chronic amphetamine group (Fig. 4a), possibly reflecting that stereotypy scores reached an asymptote in all animals of this group or that the down-regulation of Fos expression in the matrix compartment was also asymptotic in these animals (as low as in controls, approximately ten cells per square

**Fig. 3.** Striosome-predominant pattern of Fos staining occurs with chronic amphetamine treatment. Photomicrograph illustrates primary location of Fos-positive nuclei (black dots) in striosome (counterstained for MOR1 as in Fig. 2) following challenge with 5 mg per kg amphetamine after 7-day chronic amphetamine and 7-day withdrawal treatment (compare with Fig. 2d). Scale bar, 100  $\mu$ m.





**Fig. 4.** Correlations between drug-induced motor stereotypy and gene-based measures of striatal activation are highest for lateral ISMP values for the three drug treatments examined. Vertical axes in (a–d) indicate stereotypy values. Horizontal axes indicate, for rostralateral striatum, (a) ISMP value, (b) overall density of gene expression, (c) density of gene expression in the matrix and (d) density of gene expression in the striosomes. Spearman correlation values ( $r_s$ ) are indicated for each measure of striatal function. Cocaine, gray triangles; amphetamine, white squares; and apomorphine, black circles.

millimeter). In the presence of an active matrix, as occurred in the chronic cocaine group, relatively small increases in the ISMP were associated with pronounced changes in behavior (Fig. 4a). In the absence of significant matrix activation, however, as in the chronic amphetamine group, even smaller striosomal activation (130–270 cells per  $\text{mm}^2$ ) were associated with maximal levels of stereotypy. In this case, further increases in the activity of the striosomes might not have had a significant impact on motor behavior.

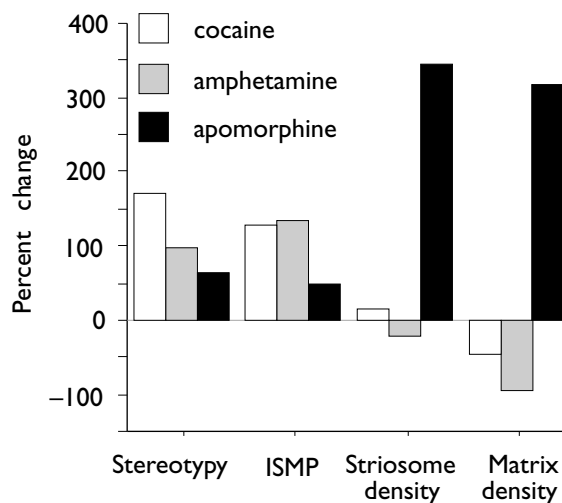
For all three drugs, increases in stereotypy induced by the treatments were paralleled by corresponding increases in ISMP values (Fig. 5), but such consistent changes were not induced in either striatal compartment considered by itself. For example, the apomorphine and chronic cocaine treatments increased striosomal activation, but the chronic treatment with amphetamine reduced it, as judged by the gene induction assay. Conversely, the activation of the matrix was reduced with the chronic cocaine and amphetamine treatments, but increased with the apomorphine treatment. These results suggest that the expression of drug-induced motor stereotypy is reflected in a pattern of relative striosomal activation, but not simply in the activation of one compartment or the other.

Previous studies suggest that dynorphin regulation in the striatum may be important for the long-term effects of exposure to psychomotor stimulants<sup>25,26</sup>. We found that the majority of Fos-positive striatal neurons also expressed dynorphin when amphetamine or cocaine was given acutely (93% and 85%, respectively) or chronically (97% and 90%, respectively). However, chronic cocaine treatment produced a 55% increase in the numbers of dynorphin-positive neurons in the caudoputamen relative to values for acute cases, whereas chronic amphetamine treatment elicited only a 6% increase. The common patterns of increased stereotypy and ISMP values that we observed thus did not seem to depend exclusively on overall dynorphin regulation in the caudoputamen, at least as judged by cell counts of dynorphin-immunoreactive neurons.

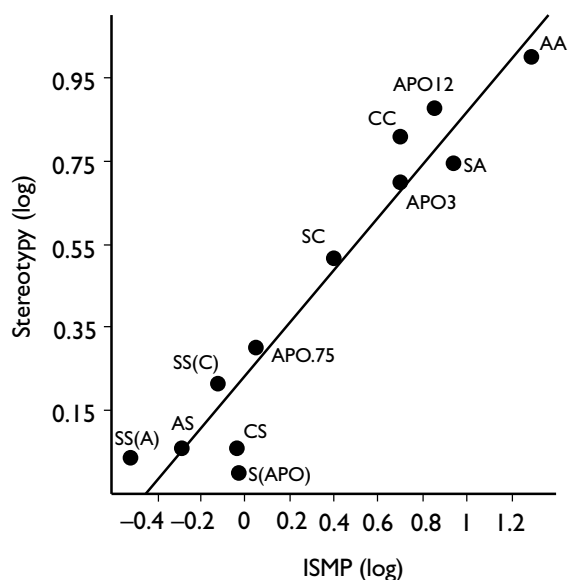
Because striatal interneurons are involved in neural plasticity<sup>21,27,28</sup>, we carried out a similar analysis with interneu-

ronal markers. The one change that we observed after the chronic treatments was an increase in the percentage of Fos-positive neurons that expressed NADPH-diaphorase, a marker for striatal interneurons containing nitric oxide (NO) synthase (3.5-fold for amphetamine and 5-fold for cocaine compared with values for acute cases). Alterations in interneuron functions in the striatum could contribute to the emergence of the striosome-predominant pattern induced by chronic drug exposure.

The induction of early genes by psychomotor stimulants requires glutamate receptor activation and corticostriatal input<sup>29–31</sup>, suggesting that the changes that we observed in the caudoputamen could reflect alterations in cortical function or corticostriatal transmission. This possibility was not directly tested in our experiments, but we did find that all of the drug treatments produced sharp increases in early gene expression in the somatosensory cortex, which projects to the matrix compartment. By contrast, we found no marked changes in the striosome-projecting orbital and medial prefrontal cortices. Changes in relative activation of cortical inputs to the matrix and striosomes could contribute to the compartment-selective effects that we observed in the striatum.



**Fig. 5.** Close parallels between stereotypy scores and ISMP values occur despite different combinations of up- and downregulation of gene expression in the individual striosome and matrix compartments. Bars, coded for treatment, indicate the percent of increase or decrease in motor stereotypy, ISMP values, density of gene expression in striosomes and density of gene expression in the matrix elicited by chronic treatments compared to acute treatments with cocaine and amphetamine and by the high dose compared to the middle dose of apomorphine.



**Fig. 6.** Correlations between averaged ISMP values and stereotypy values are high for each of the experimental treatments studied. The logarithmic transformation of averaged ISMP values and stereotypy scores provided the best fit for the cocaine, amphetamine and apomorphine data combined ( $r_s = 0.88$ ). SS(C), chronic saline-saline control for cocaine cases; SS(A), chronic saline-saline control for amphetamine cases; S(APO), saline controls for apomorphine experiment; SC, chronic saline-cocaine; CS, chronic cocaine-saline; CC, chronic cocaine-cocaine, SA, chronic saline-amphetamine; AS, chronic amphetamine-saline; AA, chronic amphetamine-amphetamine; APO.75 (0.75 mg per kg apomorphine); APO3 (3 mg per kg apomorphine); APO12 (12 mg per kg apomorphine).

## DISCUSSION

Our experiments demonstrate that a gene-based measure of neuronal activation in the two main compartments of the striatum can be used to predict the levels of motor stereotypy induced by two different psychomotor stimulants and by a direct dopamine receptor agonist. The measure of neuronal activation that we used in these experiments was at the level of gene expression rather than spike-discharge activity. The relationship between these measures is unknown<sup>32,33</sup>. The early-gene assay, however, is a useful indicator of activity in neural systems ranging from the hippocampus, visual cortex, hypothalamus and basal ganglia in mammals<sup>34</sup> to the song system in birds<sup>35</sup>. Here the gene-induction assay proved sensitive enough to detect specific compartmental activation patterns in the striatum. Enhanced activity of striosomes relative to activity of the matrix, as estimated by the striosomal predominance (ISMP) values calculated, was tightly associated with the expression of increased drug-induced stereotypy for all three drug treatments (Fig. 6). This tight association held for the rostralateral striatum despite the fact that the three drugs evoked different patterns and amounts of early gene induction in the striatum, elicited different motor responses and levels of motor stereotypy and act by different cellular mechanisms to stimulate the dopamine system. The predictive power of the ISMP values therefore was neither drug-specific nor dependent on the specific levels or type of motor responses emitted, and did not reflect a common initial mechanism of drug action. Rather, the increase in ISMP values suggested a systems-level change in

basal ganglia-based neural circuits controlling the integration and switching of motor responses in a serial fashion, mechanisms affected critically in stereotypy.

Our observations specifically point to a functional imbalance between the striosome and matrix compartments as a potential correlate of motor stereotypy. Matrix-based circuits link the striatum with sensory, motor and associative areas of the neocortex. Striosome-based circuits include cortico-basal ganglia loop circuits interconnecting the striatum with medial prefrontal cortex and orbital prefrontal cortex<sup>36,37</sup>, regions that are disordered in stimulant-induced syndromes in humans and other primates<sup>38</sup> and, in humans, in neuropsychiatric disorders such as obsessive-compulsive disorder. Striosomes are thought to project to the pars compacta of the substantia nigra or its immediate vicinity<sup>36,37</sup> and could have a strong regulatory influence on the dopamine system. The effects that psychomotor stimulants and direct dopamine receptor agonists have in disrupting the orderly integration of adaptive motor behaviors, a key feature of stereotypy, are exerted through their actions on such dopamine-modulated basal ganglia circuits<sup>39,40</sup>.

As purely correlational findings, our results leave open the question of how general the relationship is between the induction of motor stereotypy and patterns of relatively increased striosomal activation. In accord with our observations, combinations of dopamine D1-class and D2-class receptor agonists produce intense stereotypies as well striosome-enhanced gene expression<sup>41–43</sup>. In mutant mice lacking dopamine D1 receptors, psychomotor stimulants fail to induce motor stereotypies, and these mice have severe deficits in the striosome-enhanced neuropeptide dynorphin and do not show stimulant-induced Fos expression<sup>21,44,45</sup>. Conversely, dopamine D2-class receptor antagonists such as typical neuroleptics can block apomorphine-induced stereotypy and can activate early genes in non-compartmental patterns emphasizing enkephalinergic neurons<sup>46–48</sup>.

Our observations also leave open the question of how other behaviors relate to the ISMP ratios. For example, locomotion and rearing in rodents are known to compete with stereotypies for expression, and might actually be negatively correlated with the ISMP ratios. Further, behavioral stereotypies are probably mediated by complex neural mechanisms, but we have quantified neuronal responses only in the striatum and with only one neuronal measure. Even so, the strength of the correlations that we did find suggests that the dopamine-modulated pattern of relative striosome-matrix activation of striatal circuits is an important factor contributing to the expression of stereotypy.

The basal ganglia are implicated in the release of specific motor acts and complex behaviors<sup>49,50</sup>. Imbalances between the direct and indirect pathways of the basal ganglia are proposed as leading to hypokinetic syndromes such as Parkinson's disease and hyperkinetic syndromes such as Huntington's disease<sup>49,50</sup>. The release of movement is, by the same theory, thought to be achieved by a balance between these two sets of pathways<sup>49,50</sup>. Our findings suggest that the basal ganglia have, in addition to a mechanism controlling the release or inhibition of movement, compartmentally organized circuits that can regulate the frequency of movement release and can mediate response selection. Imbalances in the relative activity of such striosome-based and matrix-based circuits might produce changes in the selection and release of behavioral alternatives, thereby producing inflexible behavior and stereotypy at one extreme and poverty of behavior at the other.

## METHODS

**Treatment protocols.** Male Sprague-Dawley rats (250–350 g) were treated with procedures approved by the Massachusetts Institute of Technology Committee on Animal Care. Cocaine hydrochloride and amphetamine sulfate (Sigma) dissolved in 0.9% saline were administered i.p. at doses of 25 mg per kg and 5 mg per kg, respectively. Doses were based on dose-response studies described elsewhere<sup>21</sup>. Rats ( $n = 55$ ) were given daily injections of amphetamine, cocaine or saline at 10:00 AM and 5:00 PM for 1 week, followed by 1 week of drug withdrawal. On day 15, each rat was challenged with amphetamine, cocaine or saline. Apomorphine (Sigma) dissolved in 0.1% ascorbic acid was acutely administered i.p. at doses of 0, 0.75, 3 and 12 mg per kg ( $n = 26$ ). Each rat was deeply anesthetized 1 h after the last injection with sodium pentobarbital (Nembutal; 25 mg per kg) and perfused transcardially (4% paraformaldehyde in 0.1 M NaKPO<sub>4</sub>).

**Immunohistochemistry.** Free-floating 24  $\mu\text{m}$ -thick sections were treated by standard single-antigen immunohistochemical methods<sup>21</sup> with antisera raised against cFos (Ab-5, Oncogene Science, Manhasset, New Jersey; 1:40,000), the M peptide sequence of Fos/Fra family members (Fra, M. Iadarola, NIH, Bethesda, Maryland; 1:10,000), or JunB (R. Bravo, Bristol-Myers Squibb, New Jersey; 1:10,000), and by dual-antigen immunohistochemical methods<sup>21</sup> with Ab-5 anti-Fos and antisera against the C-terminal peptide of the mu opiate receptor as a marker of striosomes (MOR1, R. P. Elde, University of Minnesota, Minneapolis, Minnesota; 1:100,000), or dynorphin (leuorphine, S. Watson, University of Michigan, Ann Arbor, Michigan; 1:10,000), choline acetyltransferase (ChAT, Chemicon, Temecula, California; 1:1,000), somatostatin (Fitzgerald Industries, Concord, Massachusetts; 1:100), parvalbumin (Sigma, 1:1,000) or calretinin (Swant, Bellinzona, Switzerland; 1:1,000) as markers for striatal neuron subtypes. NADPH diaphorase histochemistry was used to mark nitric oxide synthase-containing neurons<sup>21</sup>.

**Behavioral analysis.** Behavioral responses were monitored systematically after acute injections and after the 10 AM daily injections and the final challenges (amphetamine, cocaine). A time-sampling procedure based on the Creese and Iversen stereotypy scale<sup>8</sup> was used to score motor stereotypies. Each animal was observed twice for 1 min, at 20 min and 50 min after the injection, and the average of the 2 scores given was considered as the overall session score. The observer was blind to the experimental conditions. Scores were based on estimates of four behavioral dimensions: the number of alternative motor responses emitted (repetitiveness/flexibility), the number of responses per unit time (frequency), the percentage of time spent performing the most dominant responses (duration) and the level of spatial confinement of the motor response (spatial distribution). Motor responses examined included head (head swaying, head bobbing) and limb (forelimb, hindlimb) movements, stereotyped sniffing, grooming and rearing and oral stereotypies (licking, nibbling).

**Data analysis.** For each stain, all sections were analyzed by microscopy and counts of Fos-positive nuclei were made at as nearly as possible standard levels through the rostral (+1.6 to +1.8 mm relative to bregma) and mid-level (0.0 to +0.2 mm relative to bregma) caudoputamen of each rat. For each section, a 3.5 mm<sup>2</sup> area of the caudoputamen was sampled in medial and lateral halves with the aid of a computerized imaging system (Biocom, Les Ulis, France) as described previously<sup>21</sup>. Counts were rechecked in blind recounts of a sample of the sections. For each region, we estimated the density of Fos-positive nuclei in the matrix and in the striosomes and calculated an ISMP value (the ratio between striosome and matrix gene-expression densities). The gene expression data were analyzed by analysis of variance followed by *post-hoc* comparisons. Behavioral data were analyzed with the Kruskal-Wallis test. Correlations were calculated according to the method of Spearman ( $r_s$  values).

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