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Expression of *c-fos* mRNA in the Basal Ganglia Associated with Contingent Tolerance to Amphetamine-Induced Hypophagia

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Abstract

Tolerance to the hypophagic effect of psychostimulants is contingent on having access to food while intoxicated. Rats given chronic injections of such drugs with access to food learn to suppress stereotyped movements, which interfere with feeding. In contrast, controls given the drug after food access do not learn to suppress stereotypy and, therefore, do not become tolerant. To determine the role of the basal ganglia in this phenomenon, we used *in situ* hybridization to measure the expression of *c-fos* mRNA, a marker for neural activation, in the brains of tolerant and nontolerant rats. Rats given chronic amphetamine injections prior to food access learned to suppress stereotyped movements, whereas yoked controls given the drug after feeding did not. Following an acute injection of amphetamine, both of these groups had higher levels of *c-fos* mRNA than saline-treated controls throughout the striatum, in the nucleus accumbens core, the ventral pallidum and layers V–VI of the motor cortex. In contrast, tolerant rats, which had learned to suppress stereotypy, had higher levels of *c-fos* mRNA than both amphetamine- and saline-treated controls in the entopeduncular nucleus, globus pallidus, subthalamic nucleus, pedunculopontine nucleus, nucleus accumbens shell, olfactory tubercle, somatosensory cortex, and layers II–IV of motor cortex. These data suggest that the learned suppression of amphetamine-induced stereotypy involves the activation of dorsal striatal pathways previously implicated in response selection as well as the ventral striatum, long implicated in appetitive motivation and reinforcement.

Keywords

amphetamine; basal ganglia; contingent tolerance; *c-fos*; stereotypy; dorsal striatum; ventral striatum; response selection/suppression

1. Introduction

In addition to being potent drugs of abuse, psychostimulants induce locomotion and stereotyped movements and suppress food intake. There is increasing evidence that the environmental context in which these drugs are administered exerts a profound influence on the expression of these behavioral effects [6,50]. For example, the increase in locomotion

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induced by acute administration of amphetamine is greater when the drug is administered in a novel environment than when it is given in the home cage [7,8]. Similarly, sensitization of locomotion following chronic administration of psychostimulants is greater when the drug is administered in a novel environment [7,8].

Environmental context also plays a critical role in the development of tolerance to the hypophagic effects of psychostimulants [60]. In this case, tolerance is contingent on experiencing the drug in an environment containing food. Rats given psychostimulants and access to sweetened milk develop tolerance to drug-induced hypophagia, whereas rats given the same number of injections *after* they have had access to milk do not [12,16,25]. Such “contingent tolerance” is mediated by the learned suppression of stereotyped head movements, which interfere with feeding. When behavioral interference from stereotypy is bypassed by delivering milk directly into the mouth through intraoral cannulas, rats show little hypophagia to psychostimulants [61,64,66]. This demonstrates that food remains reinforcing and, therefore, can serve as an incentive for learning to suppress stereotypy. Indeed, amphetamine-treated rats learn to maintain a stationary head position when reinforced with intraoral infusions of milk and they do so at the same rate at which bottle-fed rats develop tolerance [65]. Remarkably, rats learn to suppress stereotyped movements even after sensitization develops [61,62,63].

Little is known about the neural mechanisms underlying contingent tolerance to amphetamine. Understanding the neural basis of this phenomenon would broaden our understanding of how environmental context modulates psychostimulant drug effects. Moreover, amphetamine-induced stereotypy in rodents is often used to model dyskinesias and tics in humans [14,25]. These involuntary movements can be at least partially suppressed by reinforcement-based behavioral therapies, such as “habit reversal” [4,45,58]. Thus, understanding the neural mechanisms underlying the learned suppression of stereotypy may have clinical relevance as well.

Previous research has established that stereotypy involves the dorsal striatum and its output pathways (see Discussion), whereas the reinforcing effects of both natural rewards and psychoactive drugs involves the ventral striatum and associated structures [33,34]. It seems likely, therefore, that the learned suppression of stereotyped movements involves changes in the neural activity of at least some of these brain regions. As a first approach to addressing this issue, we examined levels of mRNA expression for the immediate-early gene *c-fos*, used here as a marker for neural activation, in the basal ganglia of tolerant and nontolerant rats following acute injection of amphetamine. At doses that induce stereotyped movements, amphetamine increases *c-fos* expression in the basal ganglia [15,20,26] and both the degree and the pattern of *c-fos* expression are strongly influenced by the environmental context in which the drug is given. For example, the expression of *c-fos* in both the dorsal and ventral striatum is greater if amphetamine is given in a novel environment than if it is given in the home cage [9,55]. Moreover, whereas amphetamine induces *c-fos* primarily in D1/prodynorphin-expressing neurons when administered in the home cage, it induces *c-fos* in both D1/prodynorphin- and D2/proenkephalin-expressing neurons when it is administered in a novel environment [10, 56]. Finally, environmental context influences the particular structures in which amphetamine induces *c-fos*. For example, levels of *c-fos* are increased in the subthalamic nucleus when amphetamine is given in a novel environment, but not when it is given in the home cage [56, 57]. Based on these findings, we expected that tolerant rats, which had been given injections of amphetamine in an environment containing food, would display a different pattern of *c-fos* mRNA expression within the basal ganglia than nontolerant rats given the same number of amphetamine injections in the absence of food.

2. Materials and methods

2.1. Subjects

Subjects were 46 male albino Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–275 g at the beginning of the experiment. They were housed and tested in individual stainless steel cages (17.5 cm W × 25.0 cm L × 17.5 cm H) with wire mesh fronts and bottoms in a room maintained at 24° C under a 12 hr light/dark cycle (lights on 0700 hr). The rats were fed three Purina lab chow pellets (Ralston Purina Company, St. Louis; about 15 g) and unlimited water daily. On days in which tests were not conducted, the rats were given an extra food pellet (about 5 g). Rats generally consume about 30 g of chow daily when fed *ad libitum*. Therefore, the restricted availability of chow ensured that the rats were moderately food deprived during the initial stages of the experiment, prior to the development of tolerance. All procedures were approved by the university's Institutional Animal Care and Use Committee and conformed to NIH guidelines.

2.2. Drugs

d-Amphetamine sulfate (Sigma, St. Louis) was dissolved in sterile isotonic saline and injected in a volume of 1 ml/kg. Doses of the drug are expressed as the weight of the salt. Sodium pentobarbital (Abbott Laboratories, N. Chicago, IL) was administered at a dose of 150 mg/kg. All injections were intraperitoneal.

2.3. Milk tests

In the first phase of the experiment, the rats were given injections of saline (1 ml/kg) both before and after the milk tests for 36 trials to establish a stable baseline of milk intake. Milk tests were conducted six days per week, in the morning. On test days, water bottles were removed from the cages, the rats were weighed, injected with saline, and 20 min later given Eagle Brand sweetened condensed milk (Borden, Columbus, OH) diluted with water (1:3) in calibrated drinking tubes attached to the front of the cages for 30 min. At the end of the test, intakes were recorded and the drinking tubes were removed. Approximately 30 min later, the rats were given a second injection of saline. They were then fed and their water bottles were returned.

In the next phase of the experiment, dose-response tests were conducted to assess the rats' sensitivity to amphetamine. Test doses of amphetamine (0.5, 1, 2 and 4 mg/kg) and saline were administered in counterbalanced order 20 min prior to the milk tests with at least three days between doses. On the intervening days, saline injections were given. In addition to measuring milk intake, motor activity was rated seven times during each trial: 5 min before milk access, 5, 10, 15, 20 and 25 min after milk was presented, and 5 min after the milk bottles were removed. Motor activity was assessed using a 6-point nominal rating scale, which included the following categories: 0 = stationary and immobile; 1 = stationary activity without stereotyped head movements (e.g., grooming, drinking); 2 = movement involving one or both forelimbs without concurrent stereotyped head movements (e.g., pivoting, rearing, walking); 3 = stereotyped head movements accompanied by sniffing, and generally covering a wide area; 4 = focused stereotyped head scanning movements covering a small area of the wall or floor of the cage; and 5 = stereotyped mouthing, licking, biting, or gnawing. At each rating interval, each rat was observed for about 5 s by a trained observer, who scored the dominant behavior that occurred in that interval. Following the dose-response trials, the rats were given seven additional trials with both pre- and post-test injections of saline to re-establish the baseline.

Five rats that were insensitive to the hypophagic effect of amphetamine were eliminated from the experiment at this point. The remaining rats were rank-ordered based on their milk intakes under the 2 mg/kg dose of amphetamine. Cohorts of comparably sensitive rats were then

randomly assigned to one of four groups, with the extra rat assigned to the Tolerant group (see below). During the tolerance phase of the experiment, rats in the Tolerant group were given injections of amphetamine (2 mg/kg) 20 min before the milk tests and injections of saline 30 min afterward. The dose of amphetamine was selected on the basis of previous studies on contingent tolerance to amphetamine. Rats in two pharmacological control groups (Amphet/After 1 and Amphet/After 2), were given the injections in the reverse order; i.e., saline before the milk tests and amphetamine afterward. Rats in the Saline control group were given injections of saline both before and after the milk tests. In order to control for differences in milk intakes between the groups, the intakes of all three control groups were yoked to those of the Tolerant group. This was accomplished by staggering the trials by one day, so that the latter groups were given the mean amount of milk ingested by the Tolerant group on the previous day.

During the course of the tolerance phase, three additional rats were eliminated from the experiment. Two of these rats became extremely irritable when handled and the other inexplicably stopped drinking milk. Consequently, the final composition of the groups was as follows: Tolerant (10), Amphet/After 1 (8), Amphet/After 2 (10), and Saline (10).

For the purposes of this experiment, tolerance was defined as recovery of milk intake to at least 70% of baseline levels. When an individual rat in the Tolerant group reached this level of milk intake (mean: 35 d), both that rat and one rat from each of the control groups were given a final milk test on the following day, during which milk intake was unrestricted. On this final test, rats in both the Tolerant group and the Amphet/After 1 group were given injections of amphetamine 20 min before the test. Both of these groups had identical histories of amphetamine treatment and milk intake during the tolerance phase of the experiment, although the temporal relation between these events was different. Thus, the Amphet/After 1 group served as a nontolerant pharmacological control group. Rats in the Amphet/After 2 group and the Saline group were given pretest injections of saline on the final test day. These groups controlled for the effects of having a history of amphetamine injections during the tolerance phase (Amphet/After 2 group) and for the effects of having a history of milk intake during the tolerance phase (Saline group). Thirty minutes after presentation of the milk on the final test day, the rats were euthanized with an injection of sodium pentobarbital and their brains were removed, frozen in isopentane, and stored at -70°C .

2.4. In situ hybridization

Coronal serial brain sections (1 in 7; 20 μm) were cut in a cryostat at -20°C , postfixed in 4% paraformaldehyde in 0.1M phosphate buffer (PB), collected onto charged glass slides, and stored at -20°C . Tissue was processed for *in situ* hybridization using *in vitro*-transcribed ^{35}S -labeled RNA complementary to rat *c-fos* mRNA as previously described [27]. Antisense *c-fos* RNA comprised a 667 base transcript complementary to positions 583–1250 of the clone pcfos(rat)-1 ([21]; GenBank sequence X06769). Hybridization was conducted at 60°C for 16–18 hrs with the cRNA at a final concentration of 1×10^7 cpm/ml. Posthybridization treatment included incubation with RNase A (12 Kunitz units/ml) for 30 min at 45°C . All hybridizations included brain tissue from a cohort of matched animals representing all treatment groups. Labeling specificity was verified by hybridization of some sections with ^{35}S -labeled *c-fos* sense RNA. The distribution of *c-fos* cRNA hybridization was evaluated using Kodak Biomax MR film and Kodak NTB emulsion autoradiography with exposure intervals of 13 days and 13 weeks, respectively. Tissue was stained with cresyl violet or hematoxylin for microscopic analysis. Sections were viewed and photographed with an Olympus AX70 microscope equipped with a Magnafire digital camera. Composite images were prepared in Adobe Photoshop, with adjustments made for brightness and contrast.

2.5. Densitometry

Comparisons of neural activation patterns across animals were made using film autoradiography. Autoradiograms were digitized and analyzed on a computer-based imaging system using the public domain program NIH Image v.1.62 (Wayne Rasband, NIMH, National Institute of Health Image). Images were calibrated and density measured relative to ^{14}C -labeled standards exposed to each sheet of film (American Radiolabeled Corp., St. Louis, MO). Hybridization densities in brain structures of interest were normalized to background labeling measured in white matter (corpus callosum) in the same section. Labeling density in each brain structure was sampled in a minimum of four sections per animal, with levels of section matched across animals as closely as possible. Section means were used to calculate a mean labeling density per structure (expressed as $\mu\text{Ci/g}$ of radiolabeled plastic standard), for each animal. Labeling ratios were calculated by dividing the mean density value of target structures by the value measured in white matter, and these ratios were used to generate group means.

Because this study was somewhat exploratory, our initial focus was on the basal ganglia and related brain regions. Specific neural structures examined are listed in Table 1.

2.6. Data analysis

The data were analyzed by analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison post hoc tests when significant differences were obtained. For the behavioral data, the primary dependent variable was the mean amount of milk ingested on each trial. In analyzing the activity data, the dependent measure was the frequency of each category of behavior on each trial. A composite activity score consisting of the sum of the frequencies of locomotion, sniffing, head scanning, and oral stereotypy was computed in order to provide a general index of motor activity. In presenting these data graphically, the combined frequencies of these categories of behavior were expressed as a percentage of the total number of observations from all categories (labeled “% Total Activity”). Data from the five 5-min intervals when milk was available were analyzed separately from those collected before and after milk access. Data from the latter two intervals were combined.

In analyzing the *c-fos* data, square root transformations were performed on the density ratios to control for violations of homogeneity of variance. One-way ANOVA was then performed for each neural structure, followed by Newman-Keuls multiple comparison post hoc tests. A minimum alpha level of .05 was used for all statistical tests.

3. Results

3.1. Milk intake and body weight

Amphetamine produced similar dose-dependent decreases in milk intake in all of the groups during the dose-response tests (Fig. 1). Thus, any differences in *c-fos* mRNA expression between the groups cannot be attributed to different sensitivities to the drug. As shown in Figure 2 (left), the Tolerant group showed a marked decrease in milk intake on the first tolerance trial compared to its baseline, but developed substantial tolerance by the final trial. The other groups, which received daily pretest injections of saline and restricted volumes of milk during the tolerance phase, always drank all of the milk they were offered. Thus, the yoking procedure was successful in ensuring that all of the groups had similar histories of milk ingestion during the tolerance phase. Milk intakes on the final tolerance trial, when all of the groups were given unrestricted access to milk, are presented in Figure 2 (right). Statistical analysis of the data revealed significant group differences ($F_{(3, 32)} = 6.57, p < 0.002$). Post hoc comparisons showed that pretest injections of amphetamine resulted in substantially higher intakes in the Tolerant group than in the Amphet/After 1 group, confirming the development of contingent tolerance. The Amphet/After 2 and Saline groups, which were injected with saline on the final

tolerance trial, ingested substantial amounts of milk and their intakes did not differ from each other or from that of the Tolerant group. Both of these control groups also ingested significantly more milk than the Amphet/After 1 group.

All of the groups lost weight during the course of the experiment. [means ($\pm$ S.E.M.) = Tolerant: 15.7 ($\pm$ 4.7) g; Amphet/After 1: 22.4 ($\pm$ 5.6) g; Amphet/After 2: 21.5 ($\pm$ 4.3) g; Saline: 14.7 ($\pm$ 3.7) g]. There were no significant differences between the groups.

3.2. Motor activity

On the first tolerance trial, rats in the Tolerant group displayed stereotyped movements consisting of sniffing and head scanning during 98% of the observation periods while milk was available (Fig. 3, left). On the final tolerance trial, the frequency of stereotypy decreased to 28%. In contrast, during the periods before and after milk was available, the frequency of stereotypy remained at 100% (data not shown). Rats in the Amphet/After 1 group displayed sniffing and head scanning stereotypies during 82% of the observation periods on the last tolerance trial, when they were given pretest injections of amphetamine (Fig. 3, right). Statistical comparisons of the Tolerant group on the first and last tolerance trials with the Amphet/After 1 group on the last tolerance trial yielded significant differences ($F_{(2,19)} = 21.79$, $p < 0.0001$). Post hoc analyses confirmed that the Tolerant group showed a significant decrease in composite activity on the last tolerance trial compared to the first trial. This level of activity was also significantly less than that of the Amphet/After 1 group on the last tolerance trial. The level of activity of the latter group was not significantly different from that of the Tolerant group on the first tolerance trial. Rats in the control groups, which were injected with saline prior to feeding, did not display stereotyped movements.

3.3 *c-fos* mRNA expression

Mean levels of *c-fos* mRNA in various brain regions, normalized to background labeling in the corpus callosum, is shown in Table 1. The Tolerant group expressed significantly higher levels of *c-fos* mRNA than any of the other groups in the somatosensory cortex, layers II–IV of motor cortex, entopeduncular nucleus, globus pallidus, subthalamic nucleus, and pedunculopontine nucleus as well as in the nucleus accumbens shell and olfactory tubercle. Calibrated pseudocolor images of *c-fos* mRNA expression at the level of the anterior striatum for a representative cohort of rats is shown in Figure 4 and photomicrographs showing the cellular localization of *c-fos* mRNA hybridization in the subthalamic nucleus and pedunculopontine nucleus are shown in Figure 5. Levels of *c-fos* mRNA expression in these regions in the other groups were not significantly different.

In other brain regions, levels of *c-fos* mRNA expression were higher in both the Tolerant and Amphet/After 1 groups than in the Amphet/After 2 and Saline groups. These regions included the layers V–VI of motor cortex, the striatum, nucleus accumbens core and ventral pallidum. There were no significant differences in *c-fos* mRNA expression between the Tolerant and Amphet/After 1 groups or between the Amphet/After 2 and Saline groups in these areas. Thus, these effects are associated with the administration of amphetamine on the final test day. No group differences in *c-fos* mRNA expression were found in the substantia nigra pars compacta or pars reticulata or in the superior colliculus. In the ventrolateral, ventromedial and ventral anterior nuclei of the thalamus, the Tolerant group had higher levels of *c-fos* mRNA expression than the Amphet/After 2 and Saline groups, but none of these groups differed from the Amphet/After 1 group. Finally, in the lateral hypothalamus, the only significant difference in *c-fos* mRNA expression was between the Tolerant group and the Amphet/After 2 group. A summary of the statistical results is presented in Table 2.

4. Discussion

4.1. Potential confounds

The goal of this experiment was to identify structures within the basal ganglia associated with contingent tolerance to amphetamine-induced hypophagia, using the expression of *c-fos* mRNA as a marker for neural activation. At the behavioral level, contingent tolerance is mediated by the learned suppression of stereotyped movements, a process that is reinforced by the ingestion of milk [60]. Because the transcription of *c-fos* can be induced by a wide range of variables [28], it is important to differentiate between changes in expression associated with the learned suppression of stereotypy and those associated with other variables confounded with the development of contingent tolerance. These include (1) a history of repeated amphetamine injections and milk intake during the tolerance phase; (2) the pharmacological effects of amphetamine on the final test day; and (3) drinking milk on the final test day. The experimental design of this experiment included control groups for each of these variables. Like the Tolerant group, both of the Amphet/After groups had a history of daily amphetamine injections and milk intake during the tolerance phase; the Amphet/After 1 group received an injection of amphetamine on the final test day; and both the Amphet/After 2 and Saline groups had substantial milk intakes on the final test day. Thus, if increased *c-fos* mRNA expression in the Tolerant group was caused by any of these variables, it should appear in the corresponding control group as well.

4.2. Patterns of *c-fos* expression

Analysis of the *c-fos* data revealed two patterns of neural activation, one associated with contingent tolerance and the other with the administration of amphetamine *per se*. Regarding the latter, both the Tolerant group and the Amphet/After 1 group displayed higher levels of *c-fos* mRNA than the other groups throughout the striatum, in layers V–VI of motor cortex, and in the nucleus accumbens core and ventral pallidum. There is considerable evidence that the dorsal striatum is intimately involved in amphetamine-induced stereotyped movements. Graybiel et al. [26] demonstrated that systemic injection of amphetamine and cocaine at doses that induce stereotyped movements increases the expression of immediate-early genes, including *c-fos*, in both the matrix and striosome compartments of the striatum, with a strong striosomal predominance. When the drugs are given chronically to induce sensitization of stereotypy, the degree of striosomal predominance increases, apparently due to the down-regulation of *c-fos* in the matrix. Indeed, there is a strong positive correlation between the level of stereotypy and the degree of striosomal predominance [15]. Other research has shown that depletion of striatal dopamine by 6-hydroxydopamine either abolishes amphetamine-induced stereotypy [18,19,32,47] or causes a shift to stereotyped movements associated with lower doses of the drug (e.g., from oral stereotypy to sniffing and head scanning; [24]). Conversely, infusion of amphetamine directly into the ventrolateral striatum induces oral stereotypy [11, 23,35].

In contrast, the ventral striatum has been implicated in amphetamine-induced locomotion [32,54]. Early studies linked the nucleus accumbens to locomotor-related areas of the brainstem via the ventral pallidum [41,42]. More recent studies have emphasized connections of the accumbens core and ventral pallidum with basal ganglia-related structures, including the entopeduncular nucleus, subthalamic nucleus and substantia nigra [36,67]. Infusion of 6-hydroxydopamine into the accumbens core produces a decrease in amphetamine-induced locomotion and the magnitude of the effect is correlated with the degree of dopamine transporter loss, as measured by autoradiography [13,53]. This correlation does not hold when 6-hydroxydopamine is infused into the accumbens shell, although infusion of amphetamine into both the core and the shell induces locomotion [29,30].

The fact that both the Tolerant and Amphet/After 1 groups showed comparable levels of *c-fos* mRNA in these movement-related areas while displaying markedly different levels of overt behavior appears paradoxical. We assume, however, that while the activation of these structures is involved in the initiation of movement, the “selection” of particular movements for expression and the inhibition of others is the result of neural processing “downstream,” as will be discussed below. According to this view, tolerance does not alter amphetamine’s ability to activate stereotypy-related circuits in the dorsal striatum; rather, it alters the activity of other neural circuits that inhibit the expression of those movements in the appropriate behavioral context. This interpretation is supported by the fact that tolerant rats display stereotyped movements during pauses in drinking and immediately resume such movements when milk bottles are removed from the cages at the end of the session.

A second pattern of neural activation was found only in the Tolerant group, and, therefore, may be attributed to the suppression of stereotypy. In this group, higher levels of *c-fos* mRNA were found in the globus pallidus, subthalamic nucleus, entopeduncular nucleus, pedunculopontine nucleus, somatosensory cortex, and layers II–IV of motor cortex, as well as in the nucleus accumbens shell and olfactory tubercle. These structures include major components of the dorsal striatal output pathways as well as the mesolimbic dopamine system. The nucleus accumbens shell has been implicated in appetitive motivation, particularly in relation to feeding [33], and the activation of this site presumably reflects the role of reinforcement in the learned suppression of stereotyped movements. The dorsal striatal sites have all been implicated in the expression of amphetamine-induced stereotyped movements. For example, bilateral infusions of muscimol, a GABA agonist, into the entopeduncular nucleus or subthalamic nucleus induce stereotyped sniffing, head scanning and gnawing that is indistinguishable from that induced by systemic injections of amphetamine [51]. Similarly, infusion of muscimol into the pedunculopontine nucleus blocks stereotyped sniffing and head scanning movements elicited by systemic injections of amphetamine, cocaine and apomorphine [5,17].

It should be noted that not all brain regions implicated in the expression of stereotypy displayed increased levels of *c-fos* mRNA in the amphetamine-treated groups. For example, previous research has shown that infusion of muscimol into the substantia nigra pars reticulata induces stereotyped sniffing and head scanning at low doses and oral stereotypy at high doses [44, 52]. Conversely, infusion of muscimol into the superior colliculus blocks apomorphine-induced oral stereotypy [22] and lesions of this structure block amphetamine- and apomorphine-induced oral stereotypy but not stereotyped sniffing and head scanning [46,48].

It is not clear what role the increased activity of the motor cortex played in contingent tolerance. Activation of motor cortex cannot reflect drinking responses, since the saline control groups, which also drank milk, did not show comparable levels of activation. Similarly, thalamic nuclei that project to the motor cortex (ventrolateral, ventral anterior and ventromedial nuclei), showed higher levels of activation in the Tolerant group than in the saline controls. Since the effect was limited to layers II–IV of motor cortex, the increased activity may reflect intracortical communication related to the suppression of stereotypy. The higher level of activation of somatosensory cortex in the Tolerant group may reflect the role of proprioceptive feedback in maintaining a stationary head position while suppressing stereotyped movements.

4.3. Contingent tolerance and response selection

One of the functions attributed to the dorsal striatum is the process of “response selection” [38,49], in which the nervous system, confronted with multiple behavioral opportunities, selects a contextually appropriate response (e.g., feeding) and inhibits competing behavioral responses (e.g., exploring). Mink [38] has proposed that the “direct” output pathway from the striatum to the entopeduncular nucleus and substantia nigra reticulata is involved in

activating selected responses (via disinhibition of thalamocortical and/or brainstem circuits), whereas the “indirect” output pathway through the globus pallidus and subthalamic nucleus provides inhibition of competing responses. A “hyperdirect pathway” linking frontal cortex to the subthalamic nucleus has also been implicated in response inhibition [2,3,43]. On the basis of this model, Mink [39,40] has proposed that stereotyped movements, such as the tics characteristic of Tourette’s syndrome, result from the abnormal overactivation of a focal group of striatal neurons, resulting in the “release” of repetitive, inappropriate movements that would otherwise have been inhibited.

Mink’s model provides a useful framework for interpreting the current data. According to this view, the increase in *c-fos* mRNA expression in the dorsal striatum in both the Tolerant and Amphet/After 1 groups reflects the activation by amphetamine of sites controlling stereotyped head movements. Since these responses are driven pharmacologically, and compete with more adaptive responses (drinking), they must be suppressed. The increased expression in the globus pallidus and subthalamic nucleus in the Tolerant group may reflect neural activity associated with the suppression of these movements. The activation of the nucleus accumbens may reflect the process by which the ingestion of milk reinforces the learned response.

The pedunculopontine nucleus may be a critical site for relaying the output of this system to brainstem and spinal sites that control stereotyped head movements. This nucleus receives inhibitory GABAergic inputs from the globus pallidus and excitatory glutamatergic input from the subthalamic nucleus [59]. Although it provides ascending inputs to many forebrain areas, it also has descending projections to the lower brainstem and the spinal cord [59]. Several studies have shown that destruction of the pedunculopontine nucleus by local infusion of ibotenic acid results in the intensification of stereotypy (expressed as a shift to the left in the dose-response function) induced by both systemic and intrastriatal injections of amphetamine [1,31]. This intensification is accompanied by increased dopamine efflux in the striatum [37].

These data suggest that the pedunculopontine nucleus normally plays an inhibitory role in the expression of amphetamine-induced stereotypy, perhaps in part by modulating dopamine release from nigrostriatal neurons. Increased excitatory input to this nucleus from the subthalamic nucleus may be involved in the suppression of stereotyped movements in tolerant rats. However, other data suggest that the pedunculopontine nucleus plays an excitatory role in the expression of stereotypy. As noted above, microinfusions of muscimol into the pedunculopontine nucleus blocked stereotyped sniffing and head scanning movements induced by systemic amphetamine injections [5]. This suggests that inhibition of the pedunculopontine nucleus via GABAergic inputs from the globus pallidus may be involved in the suppression of stereotypy. However, the increase in *c-fos* mRNA expression in this nucleus argues against this interpretation.

In conclusion, the present data suggest that both dorsal and ventral striatal sites are involved in the learned suppression of amphetamine-induced stereotypy, which underlies contingent tolerance to amphetamine hypophagia. The specific contribution of each of the structures requires further investigation. In addition, analysis of the cellular distribution of *c-fos* in the striatum of tolerant and nontolerant rats should be undertaken to determine whether the suppression of stereotypy is accompanied by changes in the expression of *c-fos* in specific cell types in the striosomal and matrix compartments (cf, 10,15,26,56). Finally, changes in *c-fos* mRNA expression in other parts of the brain of tolerant animals remain to be explored. Because amphetamine-induced stereotypies are often used to model human movement disorders such as dyskinesias and tics, further understanding of the neural mechanisms underlying contingent tolerance may provide additional insights into the pathogenesis and treatment of these disorders.

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Figure 1.
Acute effect of amphetamine on milk intake (means $\pm$ SEM) as a function of dose.

Figure 2. (Left): Mean milk intakes ($\pm$ SEM) of the Tolerant group during the baseline and on the first and last tolerance trials. (Right): Effect of amphetamine or saline on milk intake on the final test day (means $\pm$ SEM). The Tolerant and Amphet/After 1 groups were injected with amphetamine (2 mg/kg) prior to the test whereas the Amphet/After 2 and Saline groups were injected with saline.

Figure 3.

Frequency of stereotyped movements (expressed as a percentage of the frequencies of all categories of behavior) in the Tolerant group on the first and last tolerance trials and in the Amphet/After 1 group on the last tolerance trial.

Figure 4.

Calibrated pseudocolor images of film autoradiograms illustrating patterns of ^{35}S -labeled *c-fos* cRNA hybridization in the rat forebrain at the level of the anterior striatum. Sections were taken from a single cohort of yoked animals representing each treatment group. (A) Saline group; (B) Amphet/After 2 group; (C) Amphet/After 1 group; (D) Tolerant group. The lines in A indicate the boundaries used to divide the anterior striatum into sampling quadrants. AcbC, accumbens core; AcbSh, accumbens shell; cc, corpus callosum; M1, motor cortex; S1, somatosensory cortex; Tu, olfactory tubercle. Bar in D = 1 mm.

Figure 5.

(Top) Photomicrographs of emulsion autoradiograms showing cellular localization of ^{35}S -labeled c-fos cRNA hybridization in the subthalamic nucleus of rats from a single cohort in the Tolerant group (A, B), Amphet/After 1 group (C), and Saline group (D). Open arrows indicate examples of individual labeled cells. (Bottom) Hybridization density in the peduncolopontine tegmental nucleus of rats from a single cohort in the Tolerant group (E, F), Amphet/After 1 group (G) and Saline group (H). A and E are bright-field images of the same area shown with dark-field illumination in B and F. aq, cerebral aqueduct; ic, internal capsule; dr, dorsal raphe nucleus; pptg, peduncolopontine tegmental nucleus; st, subthalamic nucleus; tn, trochlear nucleus; xscp, decussation of the superior cerebellar peduncle. Bar = 160 μm in A–D, 1.6 mm in E.

Table 1
Expression of *c-fos* mRNA in various brain regions

Brain region	Group			
	Tolerant	Amphet/After 1	Amphet/After 2	Saline
Cortex				
Motor				
layers II–IV	4.77 (± 0.6) [*]	3.29 (± 0.4)	2.23 (± 0.3)	2.14 (± 0.2)
layers V–VI	3.28 (± 0.4) ^{**}	2.67 (± 0.3) ^{**}	1.59 (± 0.1)	1.83 (± 0.1)
Somatosensory				
layers II–IV	5.61 (± 0.6) [*]	3.14 (± 0.3)	2.72 (± 0.4)	2.63 (± 0.4)
layers V–VI	4.26 (± 0.6) [*]	2.60 (± 0.3)	2.19 (± 0.3)	2.13 (± 0.3)
Striatum				
Rostral, DL quadrant	2.22 (± 0.3) ^{**}	1.87 (± 0.1) ^{**}	1.18 (± 0.0)	1.27 (± 0.1)
Rostral, DM quadrant	2.70 (± 0.3) ^{**}	2.09 (± 0.1) ^{**}	1.23 (± 0.1)	1.31 (± 0.1)
Rostral, VL quadrant	2.11 (± 0.2) ^{**}	1.66 (± 0.1) ^{**}	1.13 (± 0.0)	1.32 (± 0.1)
Rostral, VM quadrant	2.60 (± 0.3) ^{**}	2.13 (± 0.2) ^{**}	1.23 (± 0.1)	1.41 (± 0.1)
Caudal, dorsal	2.86 (± 0.4) ^{**}	2.23 (± 0.2) ^{**}	1.27 (± 0.1)	1.30 (± 0.1)
Caudal, ventral	2.23 (± 0.3)	1.70 (± 0.2)	1.16 (± 0.1)	1.20 (± 0.1)
Entopeduncular n.	1.19 (± 0.0) [*]	1.08 (± 0.0)	1.03 (± 0.0)	1.05 (± 0.0)
Globus pallidus	2.39 (± 0.3) [*]	1.67 (± 0.2)	1.12 (± 0.1)	1.15 (± 0.0)
Subthalamic n.	1.77 (± 0.2) [*]	1.32 (± 0.1)	1.24 (± 0.1)	1.17 (± 0.0)
Substantia nigra				
Compacta	1.31 (± 0.1)	1.10 (± 0.0)	1.08 (± 0.0)	1.11 (± 0.1)
Reticulata	1.11 (± 0.0)	1.11 (± 0.0)	1.04 (± 0.0)	1.04 (± 0.0)
Thalamus				
Ventrolateral n.	2.03 (± 0.2) [†]	1.65 (± 0.1)	1.23 (± 0.1)	1.38 (± 0.1)
Ventral anterior n.	1.76 (± 0.2) [†]	1.41 (± 0.1)	1.13 (± 0.1)	1.24 (± 0.1)
Ventromedial n.	1.86 (± 0.3) [†]	1.50 (± 0.1)	1.18 (± 0.1)	1.26 (± 0.1)
Pedunculopontine n.	1.63 (± 0.1) [*]	1.16 (± 0.1)	1.05 (± 0.1)	1.08 (± 0.1)
Superior colliculus				
Superficial	1.56 (± 0.2)	1.20 (± 0.1)	1.44 (± 0.1)	1.29 (± 0.1)
Intermediate	1.54 (± 0.1)	1.23 (± 0.1)	1.33 (± 0.1)	1.26 (± 0.1)
Deep	1.28 (± 0.1)	1.06 (± 0.0)	1.12 (± 0.1)	1.03 (± 0.0)
Nucleus accumbens				
Core	2.81 (± 0.2) ^{**}	2.23 (± 0.1) ^{**}	1.56 (± 0.1)	1.59 (± 0.1)
Shell	2.43 (± 0.4) [*]	1.56 (± 0.2)	1.39 (± 0.1)	1.47 (± 0.1)
Olfactory tubercle	18.27 (± 2.6) [*]	7.98 (± 1.0)	5.12 (± 0.8)	4.57 (± 0.7)
Ventral pallidum	1.94 (± 0.2) ^{**}	1.83 (± 0.2) ^{**}	1.23 (± 0.0)	1.32 (± 0.1)
Lateral hypothalamus	1.37 (± 0.1)	1.20 (± 0.1)	1.09 (± 0.0)	1.15 (± 0.0)

Data are mean optical density measures normalized to background labeling in the corpus callosum

^{*} Significant tolerance effect (Tolerant > all other groups), $p < 0.05$

^{**} Significant amphetamine effect (Tolerant = Amphet/After 1 > other groups), $p < 0.05$

[†] Tolerant group > Amphet/After 2 and Saline groups

Table 2Results of statistical tests comparing *c-fos* mRNA expression between groups

Brain region	<i>F</i>	df	<i>p</i>
Cortex			
Motor			
layers II–IV	10.32	3,32	0.0001
layers V–VI	9.83	3,32	0.0001
Somatosensory			
layers II–IV	9.60	3,32	0.0001
layers V–VI	7.53	3,32	0.0006
Striatum			
Rostral, DL quadrant	12.21	3,32	0.0001
Rostral, DM quadrant	14.80	3,32	0.0001
Rostral, VL quadrant	10.84	3,32	0.0001
Rostral, VM quadrant	11.69	3,32	0.0001
Caudal, dorsal	13.19	3,32	0.0001
Caudal, ventral	9.92	3,32	0.0001
Entopeduncular n.	5.22	3,24	0.006
Globus pallidus	13.00	3,32	0.0001
Subthalamic n.	4.82	3,32	0.007
Substantia nigra			
Compacta	2.95	3,30	ns
Reticulata	2.16	3,30	ns
Thalamus			
Ventrolateral n.	6.67	3,32	0.001
Ventral anterior n.	5.75	3,32	0.003
Ventromedial n	4.35	3,32	0.01
Pedunculopontine n.	13.00	3,25	0.0001
Superior colliculus			
Superficial	1.62	3,24	ns
Intermediate	1.54	3,24	ns
Deep	2.16	3,24	ns
Nucleus accumbens			
Core	14.30	3,32	0.0001
Shell	5.72	3,32	0.003
Olfactory tubercle	20.21	3,32	0.0001
Ventral pallidum	7.04	3,32	0.0009
Lateral hypothalamus	3.89	3,31	0.02