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The Role of Dopamine in the Mouse Frontal Cortex: A New Hypothesis of Behavioral Sensitization to Amphetamine and Cocaine

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KARLER, R., L. D. CALDER, D. K. THAI AND J. B. BEDINGFIELD. *The role of dopamine in the mouse frontal cortex: A new hypothesis of behavioral sensitization to amphetamine and cocaine.* PHARMACOL BIOCHEM BEHAV **61**(4) 435–443, 1998.—In previous studies we demonstrated that dopamine, specifically a D_2-receptor system, in the frontal cortex of the mouse functions to inhibit the motor response elicited by systemically administered amphetamine or cocaine; the inhibition appears to be the result of the dopaminergic activation of a GABAergic system. In the present study the inhibitory role of dopamine and GABA in the cortex was investigated in animals that were behaviorally sensitized to stimulant-induced stereotypy. For these studies various dopaminergic and GABAergic drugs were injected intracortically (IC) and their effects on stimulant-induced stereotypy were compared in nonsensitized and sensitized mice. The results indicate that the dopaminergic system in the cortex of sensitized animals, in contrast to nonsensitized controls, no longer functions to inhibit the motor response to the stimulants. The change in dopaminergic function in sensitized animals appears to be the result of a qualitative change in the D_2 dopamine receptor system and not the result of a change in the associated GABA system. The loss of the inhibitory activity of dopamine in the cortex correlated with the persistence of sensitization. These results suggest a new mechanism to account for behavioral sensitization; that is, the phenomenon is the result of a loss of stimulant-induced dopaminergic inhibition of motor activity normally mediated by the frontal cortex. © 1998 Elsevier Science Inc.

Amphetamine Cocaine Mouse Motor effects Dopamine GABA Cortex Sensitization

ALTHOUGH behavioral sensitization is characteristic of exposure to many drugs of abuse, the relationship per se of the phenomenon to drug abuse is not understood. Aside from the issue of drug abuse, sensitization, like LTP and kindling, represents an example of the influence of experience on subsequent CNS function that is, thus, relatively permanently altered because of the plasticity of the CNS. In a general sense, sensitization may be viewed as a type of memory, that is, memory of the experience of a drug effect. Most studies of sensitization have focused on the psychomotor stimulants, which have been shown to produce sensitization following either repeated daily low-dose or single, relatively high-dose treatment, illustrating that the basic changes underlying sensitization are persistent and cumulative, as well as dose dependent. That sensitization to the stimulants is associated with fundamental changes in brain function is emphasized by the findings that animals sensitized to amphetamine and cocaine are concomitantly sensitized to the convulsant activity of glutamatergic drugs (26,28). In previous studies we demonstrated pharmacologically that in normal animals stimulant-induced stereotypy is dependent upon functional glutamatergic and GABAergic pathways (22,23), and that in sensitized animals the role of the glutamatergic system is altered not only quantitatively, as described above, but also qualitatively (25). The qualitative change is related to the non-NMDA glutamate system, which does not appear to participate in mediating a stimulant-induced motor response in normal mice, but becomes a part of the involved circuitry in sensitized animals. Subsequently, there have been additional pharmacological studies that have indicated that sensitization is associated with many other qualitative changes in the neuroeffector systems involved in mediating a dopaminergic motor stimulus

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in sensitized mice (5,13,14,20,22,24,25,27). Notable are the changes in the roles of the nicotinic, cholinergic, and opioid systems. In normal animals the opioid system, but not the cholinergic system, appears to participate in the neural response to the stimulants. In sensitized animals their roles are reversed; the opioid system does not appear to participate, but the nicotinic system does. The data indicate that the sensitized response is associated with changes in those neuropathways that constitute the circuit involved in transmitting a dopaminergic stimulus in the striatum to a motor stimulus in the cortex. Although these data undermine the prevailing hypothesis that sensitization is the result of an enhanced release of dopamine [for review, see (34)], they are congruent with the earlier reports that the character of the motor response in sensitized animals is altered (36–39). The behavioral observations indicate that equivalent doses of amphetamine in nonsensitized and sensitized animals produce a different temporal pattern in the motor response, suggesting that sensitization is not simply a shift to the left in the dose–response curve.

In an attempt to identify the brain structures that are involved in these qualitative changes in the stimulant response, we investigated the role of the frontal cortex. In a previous article, we demonstrated that the dopaminergic system in the cortex is normally inhibitory on the motor response to amphetamine and cocaine (21). In the present work, we have extended the study of the role of dopamine in the frontal cortex to the sensitized state, and the results describe a qualitative change in cortical function that may provide a functional basis for the enhanced stimulus response associated with sensitization.

METHOD

Experimental Animals and Drugs

Male CF-1 mice, weighing 25–30 g, were housed in groups of 15, fed ad lib, and maintained on a 12 L:12 D cycle that corresponded with the day/night cycle. *d*-Amphetamine sulfate and cocaine HCl were obtained from the National Institute on Drug Abuse (Rockville, MD); the D_2 receptor antagonist sulpiride, the $GABA_A$ receptor antagonist (−)-bicuculline methiodide and dopamine HCl from Sigma Chemical Co. (St. Louis, MO); and the $GABA_A$ receptor agonist gaboxadol (THIP) HCl, the D_1 dopamine receptor agonist (±)-SKF-38393, *N*-allyl-HCl and antagonist R(±)-SCH-23390 HCl, and the D_2 dopamine receptor agonist (±)-2-(*N*-phenylethyl-*N*-propyl)amino-5-hydroxytetraline (PPHT) HCl and antagonist S(−)-eticlopride HCl from Research Biochemicals Int. (Natick, MA). All drug solutions were prepared using sterile isotonic saline immediately prior to administration. Drug dosages for systemic administration were calculated as mg/kg of body weight and were administered intraperitoneally (IP) in a volume of 0.1 ml/20 g of body weight. Intracortical (IC) injections were bilateral; doses were expressed in μg/side. Drug weights of the salts were not corrected for the weight of the free form.

Experimental Procedures

The experiments were designed to measure inhibitory or excitatory effects of drugs that were administered IC on the motor response induced by the IP administration of the psychomotor stimulants. The studies to determine inhibitory effects were as follows: Psychomotor-stimulant doses were selected that induced stereotypy in about 80% of nonsensitized and sensitized animals. (The responses in the present work ranged from 75 to 100%.) In general, a relatively high response facilitated the identification of antagonistic effects following IC drug administration. Some of the reported responses are 100%, which raises the problem of the influence of a "ceiling" effect on negative IC drug responses. In such instances, whenever feasible, higher IC drug doses were administered in an attempt to surmount a "ceiling" effect. For amphetamine, the dose required to produce a relatively high stereotypy response in nonsensitized animals was 12 mg/kg, IP; the comparable cocaine dose was 80 mg/kg, IP. In sensitized animals, the behaviorally equivalent doses were for amphetamine, 7 mg/kg; for cocaine, 40 mg/kg, IP. Studies to assess an excitatory influence on stereotypy induced by the IC administration of drugs were conducted in animals given a dose of amphetamine or cocaine that yielded 0–20% stereotypy in the controls; the relatively low-dose response enabled the detection of an enhanced effect. In these experiments, the dose of amphetamine in nonsensitized animals was 7 mg/kg, IP and 4 mg/kg in sensitized animals. For cocaine, it was 40 mg/kg in nonsensitized and 20 mg/kg in sensitized animals.

Animals were sensitized as described previously with a single dose of amphetamine, 12 mg/kg, or cocaine, 80 mg/kg, IP (1); these doses in naive animals generally produce stereotypy in 80–100% of the animals. Sensitization was determined 24–48 h after treatment with an amphetamine dose of 7 mg/kg or cocaine, 40 mg/kg, which produces roughly equivalent effects to those obtained with the higher doses in naive animals. All of the studies were conducted between 10.00 and 15.00 h.

Motor responses were measured in terms of stereotypy, which was described previously (21). A quantal measure was used rather than a graded measure because the CF-1 mouse, in contrast to the Sprague–Dawley rat, regardless of stimulant dose, manifests only one reliable stereotypic response, which constitutes a stationary animal exhibiting repetitive head and forelimb movements, and was used, therefore, as a quantal end point to measure stereotypy. The validity of the behavioral end point as a quantitative measure of stimulant activity in mice was established by obtaining dose–response curves, which demonstrate that the effect, like most effects, is proportional to the dose [see, e.g., (21,22,26)].

Stereotypy was assessed by an observer blind to the specific treatment during a 5-min period 30 min after stimulant administration (approximate peak-effect time). The duration of action of a motor-stimulant dose of amphetamine in the mouse is about 2 h; for cocaine, about 1 h. The 5-min observation period was found to be adequate to determine the number of animals exhibiting stereotypy because, unless the animals are disturbed, they tend to remain "locked" in stereotypy, which facilitates the measurement.

For the IC drug studies, cannulae were bilaterally implanted in the cortex of pentobarbital-anesthetized mice by standard stereotactic techniques as described previously (22). The coordinates for the cortical placement of cannulae were: anterior to bregma, 1.0 mm; lateral, 2.0 mm; and ventral, 0.5 mm; the tip of the cannulae rested on the surface of the dura. At the time of the experiments, the injectors were inserted 1.0 mm below the tip of the cannulae into the frontal cortex. Placements were verified by histological examination. The rationale for the cortical placement of cannulae was determined in an earlier study and was based on IC drug effects on stimulant-induced stereotypy (19,21). Most of the reported cortical dopamine studies have focused on the prefrontal cortex of the rat; the mouse, however, does not have an anatomically defined prefrontal cortex (9), so the placement used in the following studies is in the primary motor area of the frontal cortex, and is illustrated in a previous publication (21). The placement corresponds to Fig. 22 in Franklin and Paxinos (9).

Experiments were conducted about 1 week after surgery. In selected experiments tissue damage by the IC injections was assessed visually following cresyl violet staining; and functional damage, by the administration of standard stereotypic doses of amphetamine. None of the tested cannulated animals displayed any abnormal quantitative or qualitative response to amphetamine-induced stereotypy. For the IC drug administration, the injectors were connected by polyethylene tubing (PE-20) to two Hamilton 1-μl syringes. Drugs were infused simultaneously into each hemisphere in a volume of 0.15 μl over a period of 30 s; 60 s later the injectors were removed and the obturators replaced. All IC injections were made 5 min prior to the systemic administration of amphetamine and cocaine. All animals were used only once; that is, animals were treated IC bilaterally once and challenged once with amphetamine or cocaine.

RESULTS

The data in Table 1 represent the influence of IC-administered dopamine and $GABA_A$ agonists on stereotypy evoked by the systemic administration of amphetamine. The IC drug effects were compared in nonsensitized and sensitized mice given behaviorally equivalent doses of amphetamine, 12 mg/kg, IP in the nonsensitized, and 7 mg/kg, IP in the sensitized animals. In the nonsensitized mice, IC amphetamine, dopamine, or the D_2 agonist PPHT all blocked the motor response to systemically administered amphetamine. The D_1 agonist SKF-38393, however, appears to be ineffective, at least in the dosage tested; higher doses were not investigated because of its limited water solubility. In the sensitized mouse, amphetamine, dopamine, and PPHT lose their ability to inhibit the motor response to systemically administered amphetamine. In contrast, sensitization had no effect on the inhibitory response to the $GABA_A$ agonist THIP, as the drug blocked stereotypy in both nonsensitized and sensitized animals.

The loss of the inhibitory activity of the dopamine agonists in sensitized mice was investigated in more detail with the use of dose–response curves. In Fig. 1A the inhibitory activity of IC dopamine in nonsensitized animals is illustrated by the dose–response relationship; in these experiments the inhibitory dose 50 (ID_{50}) for dopamine is 0.88 (0.83–0.93) μg/side. In the sensitized animals, however, the inhibitory effect of dopamine is gone; in these animals, the relatively high dose of 10 μg/side, or 10 times the ID_{50}, was ineffective against the response to systemic amphetamine. These results suggest that the loss of dopaminergic inhibitory activity is not simply a quantitative shift in the dose–response curve; rather, the loss appears to denote a qualitative change in the dopamine effector system, resulting in a system that is refractory to dopamine.

The dose–response curves for THIP in nonsensitized and sensitized mice are shown in Fig. 1B. As suggested by the data in Table 1, THIP is inhibitory in both nonsensitized and sensitized animals; these results are confirmed by the data shown in Fig. 1B, which indicate that the THIP ID_{50} for blocking amphetamine-induced stereotypy in nonsensitized animals is 0.24 μg/side and 0.34 μg/side in sensitized animals. These ID_{50} values are not significantly different, which reinforces the conclusion that sensitization does not affect the responsiveness of the $GABA_A$ system in the frontal cortex.

The data in Tables 2 and 3 are the results of studies designed to extend those obtained with dopamine and $GABA_A$ agonists to the effects of their antagonists in nonsensitized

TABLE 1

INTRACORTICAL EFFECTS OF DOPAMINE AND GABA AGONISTS ON AMPHETAMINE-INDUCED STEREOTYPY IN NONSENSITIZED AND SENSITIZED MICE

	Amphetamine-Induced Stereotypy	
Pretreatment	Nonsensitized* (% Stereotypy‡)	Sensitized† (% Stereotypy‡)
Drugs (IC)		
Saline control	88	100
Amphetamine (5 μg/side)	25§	88
Dopamine (5 μg/side)	25§	100
SKF-38393 (1 μg/side)	75	88
PPHT (0.2 μg/side)	13§	75
THIP (1 μg/side)	0§	13§

*Amphetamine challenge, 12 mg/kg, IP.

†Amphetamine challenge, 7 mg/kg, IP.

‡% Stereotypy is the percentage of animals displaying stereotypy in each group; $n = 8$ animals/group.

§Value significantly different from corresponding control, as determined by a χ^2-test ($p < 0.05$).

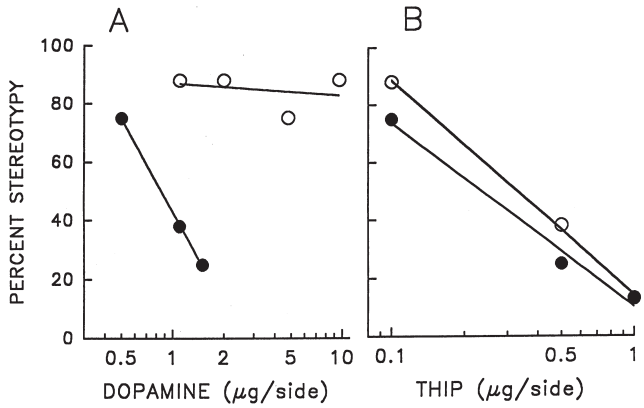

FIG. 1. (A) Comparative influence of dopamine administered IC on amphetamine-induced stereotypy in nonsensitized and sensitized mice. Nonsensitized animals are represented by ●; sensitized, by ○. Each group ($n = 8$) was challenged (29) with a behaviorally equivalent amphetamine dose; nonsensitized, 12 mg/kg, IP (controls, 88% stereotypy), and sensitized, 7 mg/kg, IP (controls, 88% stereotypy). Only the slope of the curve for the nonsensitized animals is significantly different from 0, as determined by a χ^2-test ($p < 0.05$). (B) Comparative influence of the GABA agonist THIP administered IC on amphetamine-induced stereotypy in nonsensitized and sensitized animals. Nonsensitized animals are represented by ●; sensitized by ○. Each group ($n = 8$) was challenged with a behaviorally equivalent amphetamine dose; nonsensitized, 12 mg/kg, IP (controls, 100% stereotypy), and sensitized, 7 mg/kg, IP (controls 88% stereotypy). The ID_{50} values and their 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (29). For nonsensitized animals the ID_{50} is 0.24 (0.20–0.29) μg/side; for sensitized, 0.34 (0.33–0.35) μg/side. These values are not significantly different as determined by a relative potency test ($p > 0.05$) (29).

TABLE 2

INTRACORTICAL EFFECT OF D_1 AND D_2 ANTAGONISTS ON HIGH-DOSE AMPHETAMINE-INDUCED STEREOTYPY IN NONSENSITIZED AND SENSITIZED MICE

Pretreatment	Amphetamine-Induced Stereotypy	
	Nonsensitized* (% Stereotypy‡)	Sensitized† (% Stereotypy‡)
Drugs (IC)		
Saline control	75	100
D_1 antagonist		
SCH 23390 (0.01 μg/side)	75	75
(0.1 μg/side)	0§	13§
D_2 antagonist		
sulpiride (0.01 μg/side)	88	88
(1.0 μg/side)	88	88

*Amphetamine challenge, 12 mg/kg, IP.

†Amphetamine challenge, 7 mg/kg, IP.

‡% Stereotypy is the percentage of animals displaying stereotypy in each group; $n = 8$ animals/group

§Value significantly different from corresponding control, as determined by a χ^2-test ($p < 0.05$).

and sensitized animals. These studies were divided into IC drug effects obtained in animals receiving relatively high or low doses of systemically administered amphetamine. In the high-dose studies, which are shown in Table 2, nonsensitized and sensitized controls were given behaviorally equivalent doses of amphetamine, which yielded 75 and 100% stereotypy. In these studies the D_1 antagonist, at a dose of 0.1 μg/side, blocked amphetamine-induced stereotypy in both the nonsensitized and sensitized groups. In contrast, sulpiride, the D_2 antagonist, failed to affect the amphetamine response in either nonsensitized or sensitized animals, even at a dose of 1 μg/side, which is 100 times the intrastriatal dose that blocks the amphetamine response (2).

The low-dose amphetamine study shown in Table 3 is an extension of our previous finding that sulpiride IC enhances the activity of amphetamine (21). In these experiments, the nonsensitized and sensitized groups were given relatively low behaviorally equivalent doses of amphetamine to detect an enhancement of the amphetamine effect. The data demonstrate that both D_2 antagonists, sulpiride and eticlopride, given IC to nonsensitized animals, enhance the motor response to amphetamine from 0% in the controls to 50% for sulpiride and 86% and 75% for eticlopride. The enhancement effect of sulpiride is consistent with the more detailed previous observations (21). The inhibitory role of the cortical D_2 system is further underscored by the similar effect of eticlopride, which was included in the present study to test the generality of the IC effect of the D_2 antagonists. In the sensitized animals, however, the ability of the D_2 antagonists to enhance the amphetamine response is gone. That the loss of their effect is not just a change in sensitivity to these drugs is borne out by the eticlopride data in which three times an effective dose in nonsensitized animals fails to enhance the response in sensitized animals. Higher doses of sulpiride were not tested because they cause behavioral depression, which may interfere with the motor-stimulant effects of amphetamine.

In contrast to the enhanced effect of the D_2 antagonists on the low-dose amphetamine response in nonsensitized animals, the D_1 antagonist, as shown in Table 3, is ineffective. The D_1 agonist SKF-38393 was included in this low-dose amphetamine study because in the high-dose results shown in Table 2, the D_1 antagonist inhibited the amphetamine response implying that the D_1 system may be excitatory; however the direct application of the D_1 agonist at a dose of 1 μg/side failed to enhance the response to a low-dose of amphetamine. Higher doses of the D_1 agonist were not tested because of its limited water solubility.

Like the D_2 antagonists, bicuculline, as shown in Table 3, also enhanced the effect of amphetamine in nonsensitized animals, which replicates the results reported earlier (21). The present studies, however, extend the bicuculline investigation to sensitized animals. Here, the enhanced bicuculline effect in the controls, like that of the D_2 antagonists, is absent in sensitized animals. Higher doses of bicuculline were not tested in sensitized animals because doses as low as 0.075 μg/side cause the appearance of some convulsive activity.

The disappearance of the D_2 antagonist enhancement of the effect of amphetamine in sensitized animals was examined in more quantitative detail in Fig. 2. In this figure the dose–response curve for amphetamine is shown in sensitized saline controls, and compared with the analogous curve in sensitized animals given sulpiride IC, 1 μg/side, a dose shown in Table 2 to significantly enhance the effect of amphetamine in nonsensitized animals. The effective dose 50 (ED_{50}) for amphetamine in sensitized controls was 5.3 mg/kg compared to an ED_{50} of 5.0 mg/kg in sensitized animals given sulpiride. These ED_{50} values are not significantly different, confirming that the sulpiride enhancement of amphetamine activity in normal animals described in Table 3 is absent in sensitized animals. These results are consistent with the concomitant loss of dopamine inhibition illustrated in Fig. 1A.

The data in Tables 4 and 5 represent the extension of the amphetamine studies shown in Tables 2 and 3 to a comparable investigation of cocaine. Table 4 shows the effects of IC-

TABLE 3

INTRACORTICAL EFFECT OF DOPAMINE AND $GABA_A$ ANTAGONISTS AND A D_1 AGONIST ON LOW-DOSE AMPHETAMINE-INDUCED STEREOTYPY IN NONSENSITIZED AND SENSITIZED MICE

Pretreatment	Amphetamine-Induced Stereotypy	
	Nonsensitized* (% Stereotypy‡)	Sensitized† (% Stereotypy‡)
Drugs (IC)		
Saline control	0	13
D_2 antagonist		
Sulpiride (1 μg/side)	50§	13
Eticlopride (0.01 μg/side)	86§	0
(0.03 μg/side)	75§	13
D_1 agonist		
SKF-38393 (1.0 μg/side)	0	13
D_1 antagonist		
SCH 23390 (0.1 μg/side)	0	0
$GABA_A$ antagonist		
Bicuculline (0.005 μg/side)	88§	0

*Amphetamine challenge, 7 mg/kg, IP.

†Amphetamine challenge, 4 mg/kg, IP.

‡% Stereotypy is the percentage of animals displaying stereotypy in each group; $n = 8$ animals/group.

§Value significantly different from corresponding control, as determined by a χ^2-test ($p < 0.05$).

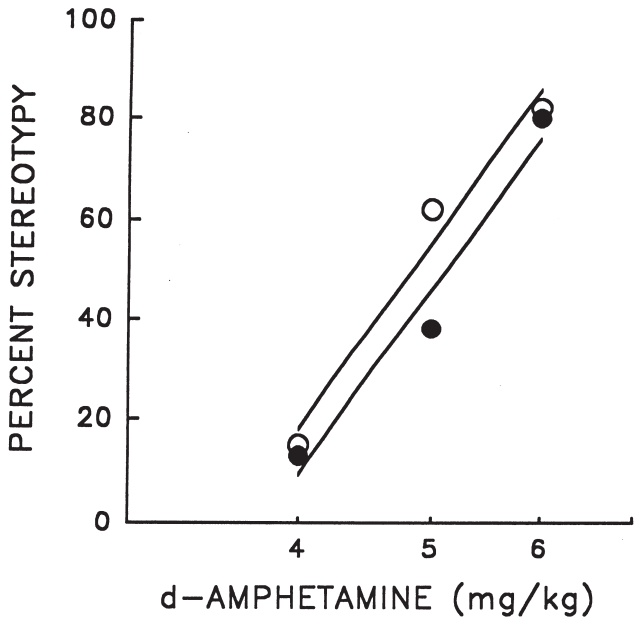

FIG. 2. The influence of IC sulpiride on the dose–response curve for amphetamine-induced stereotypy in sensitized animals. Forty-two mice were sensitized with amphetamine (12 mg/kg, IP) 48 h prior to testing. Six groups of five to eight mice were pretreated IC with either saline or sulpiride (1 μg/side) 5 min prior to varying doses of systemically administered amphetamine. The amphetamine responses in the pretreated saline controls are represented by ●; the responses in the pretreated sulpiride animals, by ○. The ED_{50} and 95% confidence limits for the vehicle pretreated are 5.3 (4.6–6.1) mg/kg; for the sulpiride pretreated, 5.0 (4.5–5.6) mg/kg. These values were calculated by the method of Litchfield and Wilcoxon (29), and the sulpiride value is not significantly different from the control, as determined by a relative potency test ($p > 0.05$) (29).

administered dopamine and GABA agonists and antagonists on high-dose, cocaine-induced stereotypy in nonsensitized and sensitized animals. The results are identical to those obtained with amphetamine; that is, in nonsensitized animals both dopamine and the $GABA_A$ agonist block cocaine-induced stereotypy, but only the dopamine effect disappears in sensitized animals. Both the dopamine and GABA antagonists were ineffective in either group of animals; again, the same result was obtained with the use of amphetamine.

Table 5 illustrates the effects of dopamine and GABA antagonists on low-dose, cocaine-induced stereotypy in non-sensitized and sensitized animals. Again, the results are identical to those obtained with low-dose amphetamine; that is, both types of antagonists enhance the activity of cocaine in nonsensitized animals, but are ineffective in sensitized animals. The cocaine data, therefore, also suggest that associated with sensitization is the loss of cortical-mediated dopaminergic inhibition of stimulant-induced motor activity.

The data in Table 6 derive from studies that were designed to assess the persistence of the loss of inhibitory activity of dopamine in the cortex. Because sensitization is characterized by its persistence, the objective of these experiments was to determine if the loss of dopaminergically mediated inhibitory activity in the cortex correlates temporally with sensitization. In these experiments a large group of animals were sensitized; and at 24 h, 7 days, and 30 days after withdrawal from sensitization,

TABLE 4

INTRACORTICAL EFFECTS OF DOPAMINE AND GABA AGONISTS AND ANTAGONISTS ON RELATIVELY HIGH-DOSE COCAINE-INDUCED STEREOTYPY IN NONSENSITIZED AND SENSITIZED MICE

Pretreatment (IC)	Nonsensitized* (% Stereotypy‡)	Sensitized† (% Stereotypy‡)
Saline control	88	75
Dopamine agonist		
Dopamine (5 μg/side)	25§	88
GABA agonist		
THIP (1 μg/side)	13§	13§
Dopamine antagonist		
Sulpiride (2 μg/side)	88	88
GABA antagonist		
Bicuculline (0.005 μg/side)	100	100

*Cocaine challenge, 80 mg/kg, IP.
†Cocaine challenge, 40 mg/kg, IP.
‡% Stereotypy is the percentage of animals displaying stereotypy in each group; $n = 8$ animals/group.
§Value significantly different from corresponding control, as determined by a χ^2-test ($p < 0.05$).

individual groups of animals were tested for the influence of IC dopamine or amphetamine on the sensitized response to IP amphetamine. The drug-treatment regimen was identical to that described above; that is, 5 min after the animals were treated IC with either saline, amphetamine, or dopamine, they were challenged with a relatively low dose of amphetamine IP to evoke the sensitized response. None of the animals was used more than once. The control groups, which received saline IC, yielded a sensitized response at all three periods, which points to the persistence of sensitization. This was also true for the animals that received either dopamine or amphetamine IC, which indicates that sensitization is associated with a persistent loss of their normal inhibitory activity; therefore, the data suggest that

TABLE 5

INTRACORTICAL EFFECT OF DOPAMINE AND GABA ANTAGONISTS ON RELATIVELY LOW-DOSE COCAINE-INDUCED STEREOTYPY IN NONSENSITIZED AND SENSITIZED MICE

	Cocaine-Induced Stereotypy	
Pretreatment	Nonsensitized* (% Stereotypy‡)	Sensitized† (% Stereotypy‡)
Drugs (IC)		
Saline control	0	25
Sulpiride (2 μg/side)	88§	28
Bicuculline (0.005 μg/side)	100§	25

*Cocaine challenge, 40 mg/kg, IP.
†Cocaine challenge, 20 mg/kg, IP.
‡% Stereotypy is the percentage of animals displaying stereotypy in each group; $n = 8$ animals/group.
§Value significantly different from corresponding control, as determined by a χ^2-test ($p < 0.05$).

TABLE 6

TEMPORAL CORRELATION BETWEEN THE PERSISTENCE OF SENSITIZATION AND THE LOSS OF INHIBITORY ACTIVITY OF DOPAMINE AGONISTS ADMINSTERED INTRACORTICALLY ON AMPHETAMINE-INDUCED STEREOTYPY

	Withdrawal		
Treatment	24 h (% Stereotypy)	7 days (% Stereotypy)	30 days (% Stereotypy)
Saline IC + amphetamine IP	88	89	90
Amphetamine IC + saline IP	0	—	—
Amphetamine IC + amphetamine IP	75	90	90
Dopamine IC + amphetamine IP	88	—	80

Initially, all animals were sensitized to amphetamine; each group consisted of 8–10 sensitized animals, which received IC treatment 5 min prior to an 8 mg/kg, IP amphetamine challenge at the indicated withdrawal times from sensitization. The IC dose for amphetamine was 5 μg/side; for dopamine, 10 μg/side. The nonsensitized response to the 8 mg/kg, IP challenge was 13%. Only the amphetamine IC control is significantly different from the saline IC control, as determined by a χ^2-test ($p < 0.05$)

the loss of dopaminergic inhibition in the cortex correlates temporally with the persistence of sensitization.

DISCUSSION

In previous studies we reported that dopamine in the mouse frontal cortex of nonsensitized animals serves to inhibit the motor response to systemically administered amphetamine or cocaine (19,21). The inhibitory role of the cortical dopamine system was determined by the IC administration of either dopamine receptor agonists or antagonists. The focal application of indirect or direct agonists to the cortex inhibited the motor response to systemically administered amphetamine; whereas the antagonist, sulpiride, enhanced the motor activity of the stimulants. The effect of the agonists demonstrated that pharmacologically dopamine can inhibit the stimulant motor response; more significantly, the sulpiride enhancement of the response showed that physiologically dopamine in the cortex functions as an inhibitor. The results suggest that the dopamine, which is released in its terminal fields in the cortex by amphetamine normally serves to limit the amphetamine-induced motor response. These results are consistent with those reported earlier by Vezina et al. (47) and Duvauchelle et al. (7). That the cortex can influence striatal function has been insinuated by a variety of neuroanatomical, electrophysiological, and neurochemical studies (8,30,31,35, 46). In this regard, the psychomotor stimulants represent useful tools to investigate the nature of the dopaminergic cortico–striatal interaction because administered systemically they are not only dopaminergic in the cortex, but they are also dopaminergic in the striatum. which elicits a measurable behavioral response, thus providing a visual account of the cortico–striatal interaction.

The pharmacological demonstration of the inhibitory role of the frontal cortex is consistent with other reports that the dopaminergic system in the cortex subserves an inhibitory role in striatal function [see, for review, (6)]. Although the neurotransmitters that are instrumental in effecting cortical inhibition have not been defined, there is previously presented evidence that the $GABA_A$ system in the cortex mediates the inhibitory effect of cortically released dopamine (21), a deduction that is supported by the results of electrophysiological studies (4,10,33,35). The principal behavioral support for this conclusion derives from studies of the comparative effects of IC-administered $GABA_A$ and dopamine agonists on the motor response to systemically administered amphetamine or cocaine in the presence of either a dopamine or a $GABA_A$ antagonist administered IC (21) Both classes of agonists were inhibitory on stimulant-induced stereotypy, and the inhibitory effect of these drugs was blocked by bicuculline, the $GABA_A$ antagonist. On the other hand, sulpiride, the dopamine antagonist, blocked only the inhibitory effect of dopamine but not that of THIP, the $GABA_A$ agonist. These data imply that the inhibitory influence of dopamine in the cortex is mediated by the activation of a GABA system, which is consistent with neuroanatomical evidence that there exist in the frontal cortex dopaminergic–GABAergic synapses (4,12,40,43).

In the present study the role of dopamine and GABA in the frontal cortex was extended from the nonsensitized to the sensitized state, and the observation that sensitization, which is long lasting, is associated with a long-lasting loss in the dopaminergic inhibition normally extant in the cortex, provides either a functional explanation of, or a potential contributing factor to, the enhanced response, which is characteristic of the expression of sensitization. In addition, the data presented above support the premise that the inhibitory effect of dopamine in the cortex is produced by the dopaminergic activation of a GABAergic system, because, in these studies, IC bicuculline, like the D_2 antagonists, fails to enhance the motor response to amphetamine in sensitized animals; yet the motor response in sensitized animals remains inhibited by the IC administration of a $GABA_A$ agonist. These results are consistent with the hypothesis that sensitization is associated with a loss of the normal dopaminergic activation of a GABAergic system in the cortex.

Our data suggest that in the nonsensitized mouse the cortical dopaminergic system functions to inhibit psychostimulant-induced motor activity, possibly by inhibiting corticofugal efferents (6). Apparently, the dopaminergic inhibition is mediated by the D_2 receptor, because the direct application of a D_2 agonist inhibits and a D_2 antagonist enhances the motor response to the stimulants. Others have described a similar role for the D_2 system in the cortex (32,42). These data, however, are inconsistent with those of Vezina et al. (47), who reported that the dopaminergic cortical inhibition of stimulant-induced motor activity is not mediated by the D_2 receptor system but by the D_1 system. The reason for the disparity in the results is not clear. In the present studies, the role of the D_1 receptor system is more difficult to explain because the cortical application of the D_1 agonist SKF-38393 in either nonsensitized or sensitized animals was without any effect on the motor response to amphetamine; yet the D_1 antagonist blocked the response to amphetamine in both conditions. That the D_1 system functions independently of the D_2 system is also suggested by the sensitization data, which indicate that the D_2 inhibitory effect on motor behavior disappears with sensitization, whereas the blocking effect of the D_1 antagonist is persistent. What is not clear is the explanation for the absence of a D_1 agonist excitatory effect shown in Table 3. One explanation of these results is that in the cortex the D_1 receptor is under tonic, maxi-

mal stimulation; therefore, the addition of a D_1 agonist would be without an effect; yet a D_2 antagonist would be effective. A similar hypothesis was used to explain why certain neuroleptics that can antagonize the D_1 system enhance the cortical inhibition produced by VTA stimulation (45). Alternatively, there may be a subclass of D_1 receptors in the cortex that is antagonized by SCH-23390 but not activated by SKF-38393. It is clear that the question of receptor participation in the mediation of cortical effects of dopamine is complex (11,41,42,45), an observation also suggested by the reports that, depending on the level of cortical dopamine, the D_1 receptor can be either excitatory or inhibitory (48); the nature of the response may depend on interactions between the receptor, multiple ionic channels, and afferent input to the pyramidal efferents (49). What is clear, however, from the results of the behavioral effects of the D_1 and D_2 antagonists in the present report is that in the cortex these two systems relative to the psychomotor stimulants function differently—the D_1 system may be excitatory, while the D_2 system is inhibitory—and that the inhibitory function of the D_2 system is lost with sensitization.

The studies described above focus on the inhibitory role of the dopamine and GABA systems in the cortex; however, the data do not rule out the possible involvement of other neurotransmitters in the inhibitory pathway in the cortex. There are, for example, electrophysiological data that suggest that both the noradrenergic and serotonergic systems can inhibit the corticofugal output (44). Whether these systems are also involved in mediating the observed dopaminergic inhibition in the cortex remains to be determined.

The loss of the inhibitory function of the D_2 system in sensitized animals represents a qualitative change in the properties of the cortical dopamine system. Other qualitative changes have also been associated with sensitization. Such changes were first described by Segal and his co-workers, whose detailed studies of the character of the behavioral response demonstrated that the temporal pattern of the motor response was altered in sensitized animals (36–39); therefore, they concluded that sensitization is characterized not only by quantitative changes but also qualitative changes in the response. These results raise the possibility that the mechanism of the motor response in sensitized animals differs from that in nonsensitized animals.

There is ample pharmacological evidence to indicate that the motor response in sensitized animals involves different neurochemical pathways from those in normal animals. A large number of drugs have been found that block the sensitized response but do not affect the nonsensitized response. These drugs include representative agents from the three major classes of calcium channel blockers (24), the non-NMDA glutamate antagonist DNQX (22,25), the protein-synthesis inhibitors anisomycin and cycloheximide (27), and the nicotinic cholinergic antagonists mecamylamine and dihydro-B-erythroidine (20). The involvement of the cholinergic system in sensitization is also supported by a dialysis study of acetylcholine efflux in the striatum (3), in which the investigators found that amphetamine had no effect on recovered striatal acetylcholine in naive rats; however, in sensitized animals amphetamine induced a substantial increase in the acetylcholine efflux. The involvement of acetylcholine in the striatum in sensitization is further confirmed by the observation that nicotinic cholinergic antagonists administered into the striatum block the induction of sensitization (20).

That a number of different types of drugs can selectively block stimulant responses in sensitized but not in nonsensitized animals is presumptive evidence that sensitization involves qualitative changes in the response system to the psychomotor stimulants. To add weight to this premise, there are also changes in the role of the opioid systems in the motor response to the stimulants. Opioid antagonists can block the stimulant-induced motor response in nonsensitized animals (13,15–18), but these drugs are ineffective in sensitized animals (13,21). Furthermore, the loss of dopamine inhibition in the cortex described in the present article represents yet another example of a qualitative change associated with sensitization. Finally, all of the drug effects described above are long lasting, and as such they correlate with the long-lasting effect of sensitization, further strengthening the association between these changes and sensitization.

The essential role of dopamine in the sensitized as well as the nonsensitized response is apparent because dopamine antagonists can completely block the stimulant-induced motor response in both groups of animals. These effects are in contrast to those obtained with the various antagonist drugs described above because they antagonize the motor activity of the stimulants only in sensitized animals. They are not only ineffective in nonsensitized animals, but they also cannot completely block the response in sensitized animals; they block only that fraction of response that derives from sensitization. The limited efficacy of DNQX (25), the calcium channel blockers (24), the protein-synthesis inhibitors (27), and the nicotinic cholinergic antagonists (20) was demonstrated with the use of dose–response curves. The differential effect of these drugs demonstrates that the behavioral response in sensitized animals represents the sum of the activation of at least two distinct neural pathways, the normal dopaminergic pathway and the dopaminergic pathway induced by sensitization. The concept of different neural pathways subserving the nonsensitized and sensitized responses has also been suggested from a study of c-*fos* mRNA expression patterns in the rat forebrain (5). These results demonstrated that sensitization is associated with changes in the pattern of c-*fos* expression in the caudate putamen, in the nucleus accumbens, and in the cerebral cortex, and thereby implied that the neurophysiological systems involved in mediating a dopaminergic stimulant response in sensitized animals differ from those in the naive animal.

The data described above represent evidence that sensitization is associated with numerous qualitative changes in brain function in response to a dopaminergic stimulus. These changes call into question the validity of the notion that sensitized response derives from an enhanced release of dopamine (34). Although the loss of dopaminergic inhibition in the cortex provides an alternative explanation to account for sensitization, what is not known is the relationship between the various qualitative changes in function and the enhanced response characteristic of sensitization. Specifically, how does the loss of dopaminergic inhibition in the cortex relate to the other qualitative changes associated with sensitization, for example, the change in the character of the locomotor response or the various pharmacological changes described above? Which changes are primary and which are secondary? From the available data, it is obvious that sensitization involves qualitative changes in many systems, and the relationship among these systems to the sensitized response remains to be established.

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