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ORIGINAL INVESTIGATION

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Environmental modulation of the response to amphetamine: dissociation between changes in behavior and changes in dopamine and glutamate overflow in the rat striatal complex

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Abstract *Rationale:* We have previously shown that environmental novelty enhances the behavioral activating effects of amphetamine and amphetamine-induced expression of the immediate early gene c-*fos* in the striatal complex, particularly in the most caudal portion of the caudate. In contrast, we found no effect of novelty on the ability of amphetamine to induce dopamine (DA) overflow in the rostral caudate or in the core of the nucleus accumbens. *Objectives:* The twofold aim of the present study was to determine the effect of environmental novelty on (1) amphetamine-induced DA overflow in the shell of the nucleus accumbens and in the caudal portions of the caudate, and (2) glutamate and aspartate overflow in the caudal portions of the caudate. *Methods:* Two groups of rats with a unilateral 6-hydroxydopamine lesion of the mesostriatal dopaminergic system received amphetamine (0.5 mg/kg, i.v.) in physically identical cages. For one group, the cages were also the home environment, whereas, for the other group, they were a completely novel environment. In vivo microdialysis was used to estimate DA, glutamate, and aspartate concentrations. *Results:* Environmental novelty enhanced amphetamine-induced rotational behavior (experiments 1–3) but did not alter amphetamine-induced DA overflow in either the shell of the nucleus accumbens (experiment 1) or the caudate (experiment 2). In addition, the ability of environmental novelty to enhance amphetamine-induced behavioral activation was not associated with changes in glutamate or aspartate efflux in the caudate (experiment 3). *Conclusions:* The present data indicate that the psychomotor activating effects of amphetamine can be modulated by environmental context independent of its primary neuropharmacological actions in the striatal complex.

Keywords Novelty · Context · Environment · Stress · 6-OHDA · Rotational behavior · Striatum · Nucleus accumbens shell · Caudate · Amphetamine · Dopamine · Glutamate · Aspartate · Rat

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Introduction

Addictive drugs can have very different effects depending on the context in which they are experienced (Kelleher and Morse 1968; Zinberg 1984; Barrett 1987; Falk and Feingold 1987). Little is known, however, about the neurobiological mechanisms by which pharmacological and non-pharmacological factors interact. To explore this issue, we have recently developed an animal model to compare the effects of amphetamine in rats given the drug in their home cage versus rats treated in a novel environment. Both the acute psychomotor effects of amphetamine and the development of amphetamine sensitization are enhanced when the treatment is administered in association with environmental novelty (Badiani et al. 1995a, 1997, 1998). This effect is particularly dramatic when low doses of amphetamine are administered intravenously using a remotely controlled infusion pump, a procedure that minimizes the level of arousal in the animals treated in the home cage (Crombag et al. 1996; Browman et al. 1998; Fraioli et al. 1998).

In an initial investigation of the neural bases of this phenomenon, we found that environmental novelty greatly enhances the ability of amphetamine to induce the expression of the immediate early gene c-*fos* throughout the striatal complex and particularly in the most caudal portion of the caudate (Badiani et al. 1998). There was, however, no effect of environmental context on the ability of amphetamine to induce dopamine (DA)

overflow in the rostral caudate or in the nucleus accumbens (NAcc) (Badiani et al. 1998). This was surprising because the behavioral activating effects of amphetamine are thought to depend primarily on its ability to increase DA release in the terminal regions of the mesostriatal and mesoaccumbens DA system (Wise and Bozarth 1987; Le Moal 1995). Such a discrepancy might be due to the fact that, in our earlier microdialysis study, we sampled the rostral portion of the dorsolateral caudate nucleus and the core region of the NAcc. Indeed, there are well-documented differences between different regions of the caudate nucleus and between the core and shell of the NAcc (Heimer et al. 1991, 1995; Deutch and Cameron 1992; Zahm 1992; Zahm and Heimer 1993). Thus, it is possible that environmental novelty does affect amphetamine-induced DA release in the NAcc, but only in the shell region. Furthermore, we found that within the caudate nucleus, the interaction between novelty and amphetamine in inducing c-*fos* expression was greatest in the most caudal portions (Badiani et al. 1998, 1999), and thus it is possible that environmental context modulates DA release only at this level. The aim of experiments 1 and 2 was to determine the effect of environmental novelty on amphetamine-induced DA release both in the shell region of the NAcc and in the caudal portions of the caudate, respectively.

Our second aim was to test the hypothesis that the ability of environmental novelty to enhance amphetamine-induced c-*fos* expression in the caudate might be related to alterations in glutamatergic transmission, because c-*fos* expression in the striatum appears to depend on postsynaptic NMDA receptors (Snyder-Keller 1991; Konradi et al. 1996) and requires intact cortico-striatal projections (Cenci and Björklund 1993). Therefore, in experiment 3, we tested the effect of environmental novelty on amphetamine-induced glutamate overflow in the caudal portions of the caudate.

Materials and methods

Subjects

Male Sprague-Dawley rats, weighing 200–225 g at the beginning of the experiment, were purchased from Harlan Sprague Dawley (Indianapolis, Ind.). The rats were individually housed in rooms with a 14-h light/10-h dark cycle (lights on from 0600 hours to 2000 hours) and had ad libitum access to food and water. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, D.C.: National Academy Press, 1996).

6-Hydroxydopamine lesion

One week after their arrival in the animal colony, all rats received a unilateral 6-hydroxydopamine (6-OHDA) lesion of the mesostriatal dopaminergic pathways. The animals were pretreated with desipramine (15 mg/kg i.p.) to protect the noradrenergic terminals (Breese and Traylor 1971) and were then anaesthetized with sodium pentobarbital. Using standard stereotaxic surgery procedures, a 26-gauge stainless-steel cannula was lowered into the medial forebrain bundle (coordinates in mm from bregma: AP –3.0, ML ±1.4, DV –8.2) and 4 µl of a 2-mg/ml solution of 6-OHDA was delivered over an 8-min period.

Administration of amphetamine in animals with such a lesion results in contraversive rotational behavior. The rationale for using this preparation was twofold. First, the dose–effect curve for amphetamine-induced rotational behavior is linear over a wide range of doses (Ungerstedt and Arbuthnott 1970; Crombag et al. 1999). In contrast, there is no linear relationship between dose and amphetamine-induced locomotor activity in rats without a unilateral lesion of the mesostriatal dopaminergic system (Segal and Kuczenski 1987; Crombag et al. 1999). Second, exposure to a novel environment produces robust locomotor activation, whereas it has little effect on rotational behavior (Badiani et al. 1995a). Thus, rotational behavior is more suitable than locomotor activity for studying the interactions between the effects of amphetamine and exposure to a novel environment.

Guide cannula

Immediately after the 6-OHDA lesion, an 8-mm long 21-gauge guide cannula was lowered 1 mm below the surface skull above the posterior caudate (coordinates in mm from bregma: AP –0.4, ML ±3.2) or the shell of the NAcc (AP +1.7, ML ±0.7) on the side contralateral to the lesion. A 15-mm stainless-steel post (15-gauge tubing) and an L-shaped length of PE5 tubing were also cemented to the skull at this time. The post was used to tether the rats to a liquid swivel during in vivo microdialysis (see below), and the PE tubing served to keep the distal end of the catheter in place.

Catheter surgery

All rats received an intravenous catheter into their jugular vein using standard surgical techniques. The details concerning catheter construction and catheterization procedure have been described previously (Weeks 1972; Crombag et al. 1996). At the end of the surgery, the catheter was filled with gentamicin solution (50 mg/ml) to prevent infections. For the entire duration of the experiment (between 0830 hours and 0900 hours), the catheters were flushed once daily with 50 µl of sterile heparin solution. Catheter patency was assessed at the end of the experiment by administering thiopental (12 mg/kg i.v.). The data from rats that did not become ataxic within 5 s were excluded from the study.

Apomorphine test

Seven to ten days after the surgery, the rats received a s.c. injection of 0.05 mg/kg apomorphine in the nape of the neck and the number of rotations (complete 360° turns) was measured. The appearance of vigorous contraversive rotational behavior (>4 rotations/min) indicated denervation supersensitivity, which implies a lesion of at least 90–95% of dopaminergic terminals (Hefti et al. 1980a, 1980b). Rats that did not meet this criterion were excluded from further study.

Testing procedures

Experiment 1

The aim of experiment 1 was to study the effect of environmental novelty on amphetamine-induced DA overflow in the shell region of the NAcc. After the apomorphine screen (Day 0), the rats were assigned to either an amphetamine or an amphetamine + novelty group. The amphetamine group was housed in opaque plastic cylindrical cages (25-cm diameter, 36-cm high), equipped with a drinking tube and with ground corncob bedding on the floor. The rats were left in these cages for the entire duration of the experiment (that is, the test cages were also their „home“). From day 4, the rats were tethered via a flexible stainless-steel cable to a swiv-

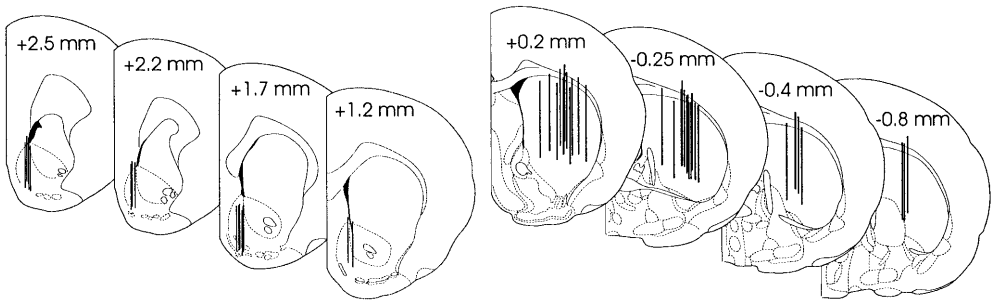

Fig. 1 Schematic drawings illustrating the placement of the microdialysis probes considered as correctly placed. This figure was obtained by digital modification of plates from Paxinos and Watson (1997)

el mounted on a counter-balanced arm located above the cage, which allowed for free movement within the cage. A 30-cm PE20 infusion line was connected at this time to the catheter and taped to the stainless-steel cable. On days 5–7, the animals underwent mock infusions to habituate them to the infusion procedures. Immediately after catheter flushing, the distal portion of the infusion lines was filled with saline solution (a tiny air bubble separated the treatment solution from the heparin solution that filled the remainder of the infusion line) and a few hours later (at 1300 hours) 50 µl heparin solution was rapidly pushed into the infusion line. Thus, each mock infusion consisted of 30 µl heparin solution (internal volume of the catheter), followed by 15 µl saline solution, and by an additional 5 µl heparin solution. At 1800 hours, the infusion lines were disconnected and the catheters flushed with heparin solution.

On the evening of day 7, a microdialysis probe (see below) was inserted into each guide cannula (aimed at the shell of the NAcc), under light ether anesthesia. A perfusion solution (145 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, and 1.0 mM $MgCl_2$) was pumped through the probes at a flow rate of 0.3 µl/min overnight. On day 8, after catheter flushing, the infusion lines were filled with 15 µl amphetamine solution. At 0900 hours, the flow rate was increased to 1.5 µl/min and, between 1130 hours and 1300 hours, three 30-min dialysate samples were collected. Heparin (50 µl) was then pushed into the infusion line, thus delivering 0.5 mg/kg amphetamine in a single bolus, and six 20-min samples were taken. Behavior was recorded using video cameras and VCR equipment, and an observer later quantified rotational behavior.

The testing procedures for the amphetamine + novelty group were similar to that for the amphetamine group, except for two major differences. On day 1, this group was housed in square cages (20.5 cm × 31 cm × 28 cm) made of transparent Plexiglas, with stainless-steel grid floors (bars 1.5 cm apart). Plastic waste trays filled with pinewood shavings were placed under the cage floors. As for the amphetamine group, the rats in the novelty and amphetamine + novelty group were tethered (day 4), underwent mock infusions (days 5–7), and received a dialysis probe aimed at the shell of the NAcc (day 7). On day 8, the infusion lines were filled with amphetamine (amphetamine + novelty group). After the third baseline sample was taken and just before the treatment was administered (at 1300 hours), the rats were transferred to test cages identical to those in which the amphetamine group lived. Thus, it is important to emphasize that although the amphetamine + novelty group received amphetamine in association with environmental novelty, the test environment (an opaque plastic cylindrical cage with ground corn cob bedding on the floor and food and water ad libitum) was physically identical to that of the amphetamine group.

Experiments 2 and 3

The aims of experiments 2 and 3 were to study amphetamine-induced DA and glutamate overflow, respectively, in the caudal portion of the caudate. In experiment 2, the rats were separated into two groups (amphetamine and amphetamine + novelty). In experiment 3, the rats were separated into three groups: amphetamine, amphetamine + novelty, and novelty. The latter group was tested as the amphetamine + novelty group but received saline instead of amphetamine. The procedures of experiments 2 and 3 were similar to those of experiment 1, except for the following differences. In both experiments, the animals received a single intravenous infusion of amphetamine and the post-treatment session lasted for 60 min. In experiment 3, the dialysis samples were collected every 10 min (five samples before the treatment and six samples afterwards).

Microdialysis probes

The microdialysis probes were similar to those described previously (Robinson and Camp 1991) with two major modifications. First, the metal shaft extended only 1 mm below bregma. Second, the dialysis portion of the probes aimed at the shell and the caudate was coated with cyanoacrylic glue except for its distal 2.5 mm (experiment 1) and 4.5 mm (experiments 2 and 3), including a 0.5-mm glue plug at the end of the probe. The probes were calibrated in vitro at room temperature to determine their ability to recover a known concentration of DA. The microdialysis probes were placed in the intact hemisphere and the final coordinates (in mm from bregma) of their tips were: AP +1.7, ML ±0.7, DV –8.5 for the shell of the NAcc, and AP –0.4, ML ±3.2, DV –7.0 for the caudate.

Histology

At the end of the experiment, the animals were deeply anesthetized with an overdose of sodium pentobarbital and perfused transcardially with 0.9% saline solution followed by 10% formalin solution. Brains were stored in a 10% formalin solution for at least 4 days and then sliced into 40-µm coronal sections. Histological verification was made on cresyl violet-stained sections with reference to the stereotaxic atlas of Paxinos and Watson (1997). The distribution of the sampling portion of the probes considered as correctly placed is shown in Fig. 1.

Quantification of DA, glutamate, and aspartate

The concentration of DA in dialysate samples was quantified using a high-performance liquid chromatography (HPLC) system coupled to an electrochemical detector, using procedures described previously (Robinson and Camp 1991). The concentrations of glutamate and aspartate in the dialysate samples were quantified using HPLC with fluorescence detection, also described previously (Xue et al. 1996; Wolf and Xue 1998). Internal standard (carboxymethylcysteine; Sigma) was added after collection, and dialysates were frozen (–80°C) for about 1 week before analysis. Precolumn derivitization with *o*-pthalaldehyde and β-mercaptoethanol was performed by an autoinjector (SIL-10 A; Shimadzu Scientific Instruments Inc., Columbia, Md.) as described by Donzanti and Yamamoto (1988). Samples in the autoinjector were maintained at 10°C by a Peltier thermoelectric sample cooler. Sample and reagent were allowed to react for 2 min. Then, a por-

tion of the mixture was injected onto a Primesphere 5-µm C18-HC column (100×4.6 mm; Phenomonex, Calif.) fitted with a Primesphere guard column (30×4.6 mm). The mobile phase was 0.1 M phosphate buffer containing 0.01 M ethylene diamine tetraacetic acid (EDTA) (pH 6.35). Acetonitrile was used as the organic eluent with a gradient profile of 13–28%. Amino acid derivatives were detected using a RF-10A fluorescence detector with excitation and emission wavelengths set at 300 nm and 400 nm, respectively. Data were taken by a PC using EZChrom 1–2 software and quantified based on peak area by comparison with standards injected throughout the run. Data were used only if alanine (which is a non-transmitter amino acid) levels remained relatively stable during the experiment (i.e., first and last samples were within 15% of the mean).

Drugs

Before surgery the animals received 0.2 mg/kg i.p. atropine methyl nitrate (Sigma Chemical Company, St. Louis, Mo.) dissolved in saline (0.5 mg/ml). Surgical anesthesia was induced with 52 mg/kg i.p. pentobarbital sodium, dissolved (64.8 mg/ml) in a 10% ethanol solution (Nembutal, The Butler Company, Columbus, Ohio), supplemented with methoxyflurane (Metofane, Mallinckrodt Veterinary, Mundelein, Ill.). 6-OHDA (2, 4, 5-trihydroxyphenethylamine hydrobromide; Sigma) and apomorphine hydrochloride (Sigma) were dissolved in a 0.9% saline/0.1% ascorbate solution (2 mg/ml and 0.1 mg/ml, respectively). Desipramine hydrochloride (Sigma) was dissolved in deionized water (0.1 mg/ml). Heparin (Sigma) was dissolved (30 USP/ml) in saline. Thiopental sodium (Pentothal, Abbott Laboratories, Chicago, Ill.) was dissolved (20 mg/ml) in saline. D-Amphetamine sulfate (Sigma) was dissolved in saline (0.5 mg/kg in 15 µl). All drug weights refer to the weight of the salts. All solutions for i.v. administration were prepared with buffered saline (0.9% NaCl) at pH 7.3.

Data analysis and statistics

Only the rats that passed the apomorphine screen and had a correctly placed probe were included in the analyses. The numbers in each group in experiment 1 were: n=5 and n=7 for the amphetamine and amphetamine + novelty groups, respectively. The numbers in experiment 2 were: n=4 and n=5 for the amphetamine and amphetamine + novelty groups, respectively. The numbers in experiment 3 were: n=7, n=6, and n=7 for the novelty, amphetamine, and amphetamine + novelty groups, respectively.

DA concentrations were corrected for probe recovery. Glutamate and aspartate concentrations were expressed as raw values. Baseline values for DA, glutamate, and aspartate concentration were calculated by averaging the values of the baseline samples and group differences were assessed using a Student's t-test. One-way analyses of variance (ANOVAs) with repeated measure (time; one level for the baseline value and one for each dialysate sample obtained after exposure to amphetamine and/or novelty) were used to assess the ability of amphetamine to alter rotational behavior and DA, glutamate, and aspartate levels in the dialysate samples. In experiments 1 and 2, group differences in rotational behavior and in DA concentrations were assessed using two-way ANOVAs with repeated measures on one factor (test environment, two levels: amphetamine and amphetamine + novelty; by time, one level for each dialysate sample obtained after exposure to amphetamine and/or novelty). In experiment 3, group differences in rotational behavior and in glutamate concentrations were assessed using a two-way ANOVA with repeated measures on one factor (test environment, three levels: novelty, amphetamine, and amphetamine + novelty; by time, one level for each dialysate sample obtained after exposure to amphetamine and/or novelty).

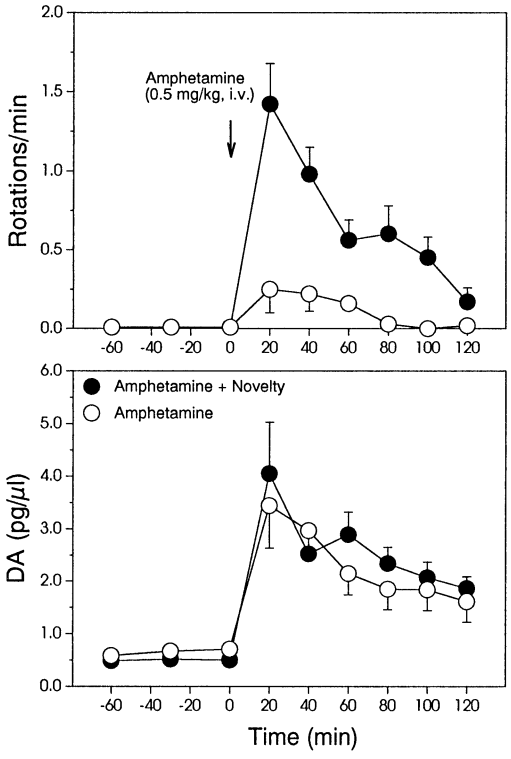

Fig. 2 Rotational behavior and dopamine (DA) concentrations in the shell of the nucleus accumbens in the intact hemisphere of animals exposed to 0.5 mg/kg amphetamine i.v., either in the home cage (amphetamine group) or in a novel test environment (amphetamine + novelty group). The *top panel* shows the mean (±SEM) number of rotations per minute before and after the treatment. The *bottom panel* shows the mean (±SEM) DA concentrations in 20-min dialysate samples from the same animals whose behavior is illustrated in the *top panel*. For the statistics see the text

Results

Experiment 1 (DA in the shell)

Figure 2, top panel, illustrates the time course of the effect of amphetamine on rotational behavior, as a function of the environment in which the treatment was administered, for rats with microdialysis probes placed in the shell of the NAcc. Amphetamine produced a significant increase in rotational behavior in both the amphetamine ($F_{6,24}$=2.94; P=0.027) and the amphetamine + novelty group ($F_{6,36}$=11.46; P<0.0001). However, this effect was much greater in the amphetamine + novelty group than in the amphetamine group ($F_{1,10}$=21.03; P=0.001; no group × time interaction, $F_{5,50}$=3.46; P=0.009).

In contrast, environmental novelty had no effect on amphetamine-induced DA release. Figure 2, bottom panel, shows the concentration of DA in dialysate samples obtained from the shell of the NAcc in the intact hemisphere for the same rats whose behavior is illustrated in the top panel. Although amphetamine increased DA concentrations in both the amphetamine ($F_{6,24}$=13.01; P<0.0001) and the amphetamine + novelty ($F_{6,36}$=9.02;

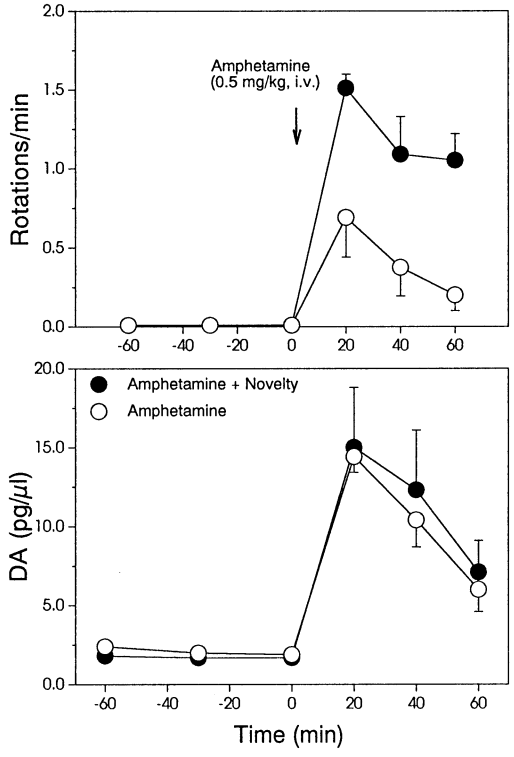

Fig. 3 Rotational behavior and dopamine (DA) concentrations in the caudate nucleus in the intact hemisphere of animals given 0.5 mg/kg amphetamine i.v., either in the home cage (amphetamine group) or in a novel test environment (amphetamine + novelty group). The *top panel* shows the mean (±SEM) number of rotations per minute before and after the treatment. The *bottom panel* shows the mean (±SEM) DA concentrations in 20-min dialysate samples from the same animals whose behavior is illustrated in the *top panel*. For the statistics see the text

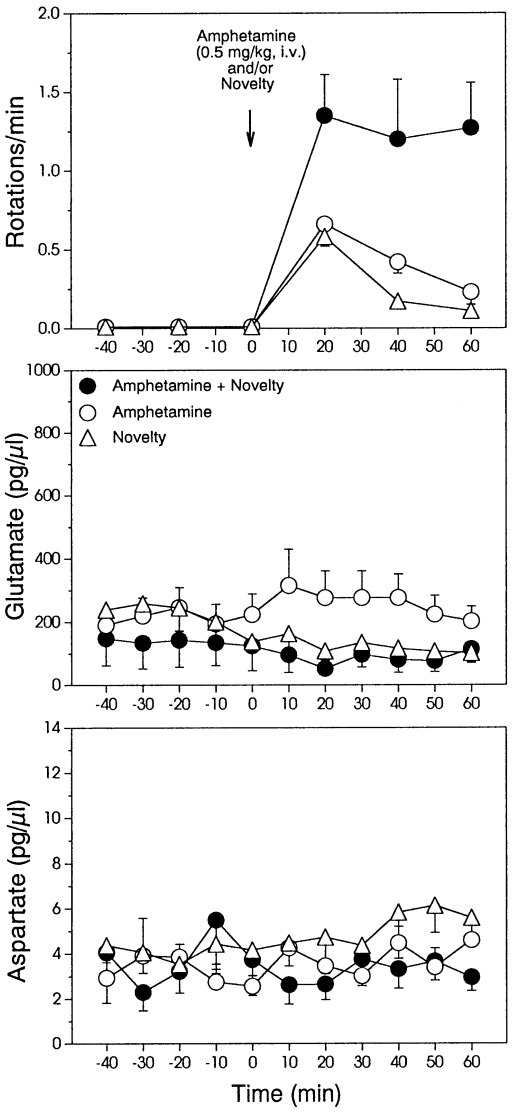

Fig. 4 Rotational behavior, and glutamate and aspartate concentrations in the caudate nucleus in the intact hemisphere of animals exposed to environmental novelty (novelty group), to 0.5 mg/kg amphetamine i.v. in home cages (amphetamine group), or to amphetamine in association with environmental novelty (amphetamine + novelty group). The *top panel* shows the mean (±SEM) number of rotations per minute before and after the treatment. The *middle* and *bottom panels*, respectively, show the mean (±SEM) glutamate and aspartate concentrations in 10-min dialysate samples from the same animals whose behavior is illustrated in the *top panel*. For the statistics see the text

P<0.0001) groups, there were no significant group differences ($F_{1,10}$=0.27, P=0.61) nor group by time interaction ($F_{5,50}$=0.84; P=0.52).

Experiment 2 (DA in the caudate)

Figure 3, top panel, illustrates the time course of the effect of amphetamine on rotational behavior, as a function of the environment in which the treatment was administered, for rats with microdialysis probes placed in the caudal caudate. Again, amphetamine produced a significant increase in rotational behavior in both the amphetamine ($F_{3,9}$=7.15; P<0.01) and the amphetamine + novelty ($F_{3,12}$=28.75; P<0.0001) groups, but there were significant differences between these two groups ($F_{1,7}$=12.09; P=0.01; no group × time interaction, $F_{2,14}$=0.23; P=0.80).

Figure 3, bottom panel, shows the time course of the concentrations of DA in dialysate samples obtained from the caudate, for the same rats whose behavior is illustrated in the top panel. Amphetamine increased DA concentrations in both amphetamine ($F_{3,9}$=45.46; P<0.0001) and amphetamine + novelty ($F_{3,12}$=11.22; P<0.0001) groups, with no significant differences between the two groups ($F_{1,7}$=0.01, P=0.76) nor group × time interaction ($F_{2,14}$=0.20; P=0.82).

Experiment 3 (glutamate and aspartate in the caudate)

Figure 4, top panel, illustrates the time course of rotational behavior in the three groups of rats with microdialysis probes placed in the caudate. Exposure to either novelty ($F_{3,18}$=8.68; P<0.001) or amphetamine (amphet-

amine group, $F_{3,12}$=11.64; P<0.001; amphetamine + novelty group, $F_{3,12}$=6.18; P<0.001) increased rotational behavior. This increase in activity, however, was much greater in the amphetamine + novelty group, as indicated by Fisher PLSD tests (P values=0.001).

Figure 4, middle and bottom panels, shows the concentrations of glutamate and aspartate in dialysate samples obtained from the caudate for the same rats whose behavior is illustrated in the top panel. Amphetamine or novelty produced no significant changes in either glutamate or aspartate concentrations in any group (all P values>0.14).

Discussion

There were three main findings in this study. First, in agreement with our previous reports (Badiani et al. 1995a, 1997, 1998; Crombag et al. 1996; Browman et al. 1998; Robinson et al. 1998), we found that exposure to a novel test environment enhanced the psychomotor activating effects of amphetamine. Second, this effect of environmental novelty was not accompanied by an increase in amphetamine-induced DA release in the shell region of the NAcc or in the caudal portions of the caudate nucleus. Third, under the conditions of the present study, amphetamine did not significantly alter glutamate or aspartate concentrations in the caudate nucleus, regardless of the environment in which the drug was administered.

Environmental novelty, amphetamine and DA release

We have previously reported that environmental novelty does not alter the ability of amphetamine to increase extracellular DA concentrations in either the rostral caudate or the core of the NAcc (Badiani et al. 1998). We now report that environmental novelty has no effect on amphetamine-induced DA overflow in the shell of the NAcc, a region functionally, neuroanatomically, and neuropharmacologically different from the core of the NAcc (Heimer et al. 1991, 1995; Deutch and Cameron 1992; Zahm 1992; Zahm and Heimer 1993). Furthermore, we found no differences in amphetamine-induced DA overflow in the most caudal portions of the caudate nucleus, in which we have previously found the greatest interaction between novelty and amphetamine for the induction of c-*fos* expression (Badiani et al. 1998).

Taken together, these findings suggest that environmental novelty does not potentiate the effect of amphetamine on rotational behavior by modulating its primary neuropharmacological action in the striatal complex, that is, by increasing DA release (Seiden et al. 1993). This conclusion might be regarded as somewhat surprising because it is often assumed that both spontaneous and drug-induced psychomotor activation are related to synaptic concentrations of DA in the striatum (Wise and Bozarth 1987; Le Moal 1995). However, our data are consistent with the report by Bardo and colleagues (1990) that the enhancement in locomotion seen when amphetamine is administered in an unfamiliar environment is not coupled with changes in DA synthesis or metabolism. Further evidence in support of the notion that the psychomotor activating effects of amphetamine are not a simple function of its ability to increase DA release in the striatum has been reviewed by Segal and Kuczenski (1994).

In previous studies, we found that environmental novelty does not only increase the acute psychomotor activating effects of amphetamine, but also enhances the development of amphetamine sensitization (Badiani et al. 1995a, 1995b, 1997; Crombag et al. 1996; Browman et al. 1998). Among the neural correlates of amphetamine sensitization, an augmentation in amphetamine-induced DA overflow in the NAcc has been reported (Robinson et al. 1988). The findings illustrated here do not exclude that novelty might enhance sensitization by increasing the effects of amphetamine on DA release. Indeed, we have found that the ability of novelty to facilitate amphetamine and cocaine sensitization is independent of its effect on the acute response to the same drugs (Badiani et al. 1995a, 1995b). Furthermore, there is some evidence that different mechanisms may be responsible for the effects of amphetamine on NAcc DA in sensitized versus non-sensitized animals. For example, the increase in NAcc DA response to acute amphetamine is a calcium-independent process, whereas the sensitization-related enhancement of this response appears to be calcium dependent (Warburton et al. 1996). Thus, the possibility that novelty enhances amphetamine sensitization owing to its ability to facilitate the effects of amphetamine on DA release remains to be tested.

Environmental novelty, amphetamine, and glutamate release

Previous studies, conducted in animals habituated for some time to the microdialysis cages (therefore similar to our home animals), have shown that only neurotoxic regimens of amphetamines (i.e., those that produce striatal DA depletion) are associated with increased striatal glutamate efflux (Nash and Yamamoto 1992, 1993; Abekawa et al. 1994; Stephans and Yamamoto 1994; Reid et al. 1997; for a review, see Wolf 1998). For example, 9.2 mg/kg amphetamine fails to alter striatal glutamate efflux or produce neurotoxicity, whereas both occur when this dose is co-administered with an inhibitor of amphetamine metabolism (Nash and Yamamoto 1993). Our study indicates that environmental novelty, either alone or in association with amphetamine, had no significant effect on glutamate efflux in the caudate. This suggests that environmental novelty does not modulate amphetamine-induced c-*fos* expression in the caudate (Badiani et al. 1998, 1999) via glutamatergic afferents from the cortex.

It has been argued that glutamate levels measured by means of in vivo microdialysis might not reflect exocyt-

otic release of this neurotransmitter and, therefore, the activity of glutamatergic neurons (Timmerman and Westerink 1997). Unlike other transmitters, amino acid transmitters also subserve metabolic roles in neurons and glia and, although basal dialysate levels of monoamine transmitters are almost completely eliminated by reducing extracellular calcium levels (Westerink et al. 1988), this reduction varies greatly depending on the brain region, the presence or absence of anesthesia, and the microdialysis protocol. Considering only those experiments using awake rats and concentric style probes, some studies have found no significant reduction in striatal glutamate concentrations upon eliminating Ca^{2+} and/or raising Mg^{2+} levels (Young and Bradford 1991; Waldmeier et al. 1992; Miele et al. 1996), whereas others have found a 20–30% reduction (Semba et al. 1995; Morari et al. 1996). The latter results are quite consistent with subcellular distribution studies showing that only 20–30% of glutamate is located in glutamatergic nerve terminals, with the remainder in the neuronal metabolic pool and glial cells (Fonnum 1985). Because of the presence of this large and unresponsive metabolic pool of glutamate, it is possible that relatively small increases in synaptic glutamate release would not be sufficient to produce a measurable increase in the extracellular compartment sampled by the microdialysis probe. Therefore, if novelty led to only a small increase in the activity of glutamatergic afferents to the caudate release, the resulting increase in extracellular glutamate levels might have escaped detection.

Conclusions

In summary, the present study indicates that the psychomotor activating effects of amphetamine can be modulated by environmental context, independent of changes in DA, glutamate, or aspartate efflux in the striatal complex (at least as measured using in vivo microdialysis). Other neurotransmitter systems not examined in the present study might be implicated in the interaction between environment and amphetamine. For example, serotoninergic mechanisms have been shown to modulate the effects of amphetamine on psychomotor activation (Gately et al. 1985; Layer et al. 1992) and striatal c-*fos* expression (Genova and Hyman 1998).

We have also considered the hypothesis that exposure to environmental novelty might enhance the effects amphetamine on behavior because of its neuroendocrine consequences. It is well known that environmental novelty produces neuroendocrine and physiological changes usually associated with conditions of stress, including the activation of the hypothalamo-pituitary-adrenal axis and corticosterone secretion (Friedman and Ader 1967; Hennessy et al. 1977; Badiani et al. 1995c, 1998), and that exposure to stress enhances the acute psychomotor response to amphetamine (Anisman et al. 1985; Williams and Barber 1989). We have shown, however, that adrenalectomy has no effect on the ability of novelty to enhance amphetamine-induced psychomotor activation (Badiani et al. 1995c). Nevertheless, it is still possible that other neuroendocrine responses to stress, independent of the secretion of adrenal hormones, such as extrahypothalamic corticotropin-releasing hormone mechanisms (Britton et al. 1986; Chappell et al. 1986; Berridge and Dunn 1989), are involved. This hypothesis remains to be tested.

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