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Modulatory Effect of Environmental Stimuli on the Susceptibility to Amphetamine Sensitization: A Dose-Effect Study in Rats[1]

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ABSTRACT

In previous studies the repeated administration of 0.5 to 1.0 mg/kg of amphetamine i.v. failed to induce psychomotor sensitization if the drug was administered to animals living in the test environment (at home). The same doses did induce sensitization if animals were transported to the test environment for each drug treatment. The purpose of the present experiment was to determine the extent to which this effect of environment is dose dependent. Rats either lived in test cages or were transported from the animal colony to test cages where they received an i.v. infusion of one of five doses of amphetamine (0.125, 0.5, 1.0, 4.0 or 8.0 mg/kg) or saline each day for 5 consecutive days. Rotational behavior was used as an index of psychomotor activation. After a 6-day drug-free period all animals were challenged with 0.5 mg/kg of amphetamine to determine the pretreatment dose necessary to induce sensitization. The effect of the drug-treatment environment was to shift the dose-effect curve for the induction of sensitization, such that significantly lower doses were necessary to induce sensitization when amphetamine was given in a novel environment. With high doses, however, sensitization occurred regardless of environmental condition. It is concluded that the circumstances surrounding drug administration can powerfully modulate the ability of psychostimulants to induce sensitization, but this effect is dose dependent.

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When psychostimulant drugs are given repeatedly and intermittently there is often a progressive and persistent increase in their ability to produce psychomotor activation, a phenomenon known as behavioral sensitization (Robinson and Becker, 1986; Stewart and Badiani, 1993). Despite considerable progress in recent years in elucidating the neurobiological basis of sensitization, the exact conditions that promote or retard the ability of psychostimulants to induce sensitization are not yet well understood. There is, however, accumulating evidence that the circumstances surrounding drug administration can powerfully modulate both the induction and the expression of sensitization (Robinson *et al.,* 1998). For example, both the acute psychomotor response to amphetamine and the rate of sensitization are greater if drug treatments are given in a relatively novel test environment than if they are given in a physically identical environment in which the animals live (Badiani *et al.,* 1995a, b, c).

This effect of environment is augmented further if most environmental stimuli predictive of drug administration (*e.g.,* presence of the experimenter, handling) are eliminated, by use of remotely controlled i.v. infusions. Thus, in the absence of stimuli predictive of drug (at home) 0.5 to 1.0 mg/kg of amphetamine i.v. failed to induce sensitization, whereas the same doses did induce sensitization if drug administration was given after transport from the animal colony and placement into a relatively novel test environment (Crombag *et al.,* 1996; Robinson *et al.,* 1998). This raises the question: is it not possible to induce behavioral sensitization with any dose of amphetamine if it is given at home in the absence of environmental cues predictive of drug administration? If the answer to this question is yes it would have important implications for how sensitization is conceptualized. For example, sensitization is often considered a nonassociative neuroadaptive process initiated by the interaction of a ligand (amphetamine in this case) and its receptor (*i.e.,* it is a primarily pharmacological phenomenon). If a simple environmental manipulation can completely prevent the induction of sensitization, at any dose, this would establish that an as yet unidentified nonpharmacological factor plays a necessary role in promoting sensitization. However, it is possible that the contribution of environment is not a necessary one, but a modulatory one. That is, an environmental factor may modulate the sensitivity of the relevant neural substrate to sensitization. In this case the effect of environment should be to shift the dose-effect curve for the induction of sensitization. Thus, the purpose of this experiment

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ABBREVIATIONS: ANOVA, analysis of variation; 6-OHDA, six-hydroxydopamine.

was to determine the extent to which environmental modulation of amphetamine sensitization is dose dependent.

Methods

Subjects

Male Sprague Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN) weighing 200 to 225 g on arrival were housed individually in wire hanging cages in the main animal colony for 1 wk before any experimental manipulation. The animal room was maintained on a 14:10 hr light:dark cycle (lights on from 06:00 to 20:00 hr) with food and water available *ad libitum.*

Surgical Procedures

Rats were anesthetized with Nembutal (pentobarbital, 50 mg/ml) supplemented with methoxyflurane, if necessary, and using standard stereotaxic surgical procedures a 21-gauge stainless steel guide cannula was placed on the dural surface above the nigrostriatal bundle in one hemisphere (for half of the animals the guide cannula was placed in the right hemisphere, and for the other half in the left hemisphere). The coordinates for the guide cannula were: posterior to bregma, −3.0 mm; lateral, ±1.8 mm; ventral, −1.0 mm from the skull surface (Paxinos and Watson, 1997). In addition, a length of 15-gauge stainless steel tubing was fixed in front of the guide cannula (to be used later as an anchor for a tether). An L-shaped piece of plastic tubing was placed posterior to the guide cannulae, and was later used to hold the end of the i.v. catheter. The entire assembly was fixed to the skull using jeweler's screws and cranioplastic cement. A stylet was inserted into the guide cannula following surgery to maintain patency.

Two to four days after guide cannula implantation all rats received a unilateral lesion of the nigrostriatal bundle via the guide cannula using the neurotoxin 6-hydroxydopamine (6-OHDA). This was done so that drug-induced rotational behavior could be used as an index of psychomotor activation. The rationale for assessing rotational behavior has been discussed in detail elsewhere (Badiani *et al.,* 1995a; Robinson, 1984). Rats were pretreated with desipramine HCl (15 mg/ml) and between 30 and 60 min later a 29-gauge injection cannula was lowered through the guide cannula into the nigrostriatal bundle. A solution of 4 μl ascorbate-saline containing 8 μg of 6-OHDA.HCl was infused over a 8-min period. After the infusion the injection cannula was left in place for 2 min before it was raised and the stylet was replaced. The animals were not anesthetized during this procedure. Two weeks after the 6-OHDA lesion animals were administered 0.05 mg/kg of apomorphine to assess the development of receptor supersensitivity. This dose of apomorphine produces robust rotational behavior only if 90 to 95% of the striatal dopamine terminals are destroyed. Ten minutes after a s.c. injection of apomorphine rotational behavior was quantified for 2 min, and animals that made less than five rotations were excluded from the study.

Animals that passed the apomorphine screen received an intravenous catheter 2 to 4 days later. The catheter and the surgical techniques were similar to those described previously (Browman *et al.,* 1998; Crombag *et al.,* 1996; Weeks, 1972). Briefly, under ether anesthesia (supplemented with methoxyflurane) one end of a catheter consisting of 0.3-mm diameter silicone rubber tubing was inserted into the right external jugular vein. The other end (PE 20 tubing) was passed s.c., exiting the skin on the nape of the neck, and was secured to the head by the plastic tubing mounted to the skull. The catheter was filled with 50 μl of a solution containing 50 mg/ml of gentamicin. A stylet was then placed in the exterior end of the catheter to maintain patency, and the animal was returned to its home cage. The animals were left undisturbed the day after surgery, and beginning 2 days after surgery catheters were flushed daily for the duration of the experiment with 0.1 ml of a heparin solution (30 USP/ml heparin in a 0.9% saline solution with a pH of 7.4).

Behavioral Testing Procedures

Group assignment and habituation. After a 4-day recovery period from the catheter implantation animals were assigned to one of two groups; which will be called the home or novel group. Animals in the home group were transported to a testing room where they were housed for the duration of the experiment. The animals were housed in circular plastic buckets that had a diameter of 25 cm at the base, ground corn cob bedding on the floor and food and water available *ad libitum.* In addition, white noise was present continuously to mask extraneous sounds. During the entire experiment these rats were tethered to a liquid swivel (modified from Brown *et al.,* 1976) via a lightweight flexible cable secured to the post mounted on their skull. The swivel was fixed to a counter-balanced arm suspended above the center of the bucket. Animals in the novel group were housed in wire-hanging cages in the main animal colony for the duration of the experiment.

The first 5 days after group assignment served as a habituation period. During this period the experimenter entered the room where the home group was housed, between 0800 and 0900 hr each day, and each rat's catheter was manually flushed with 0.1 ml of the heparin solution described above. Then the catheter was attached to one end of an infusion line consisting of a length of PE 20 tubing, the other end of which was attached to the liquid swivel, which in turn was attached (again by PE 20 tubing) to a syringe mounted on a syringe pump. A total of 40 μl of the infusion line closest to the catheter was filled with saline, and the rest of the line was filled with the heparin-saline solution. After all of the animals were attached to infusion lines, the experimenter left the room and did not return until the end of the day. The syringe pumps were accessible via a remote controlled switch, located outside the room, and at either 1100, 1300 or 1500 hr the pump was activated to deliver a total volume of 80 μl over a 4-min period at a rate of 20 μl/min. Thus, this procedure served to habituate animals to the infusion procedure (see below). Between 1700 and 1800 hr each animal was disconnected from the infusion line, and a stylet was reinserted into the catheter. During the habituation period animals in the novel group had their catheters flushed once a day with heparin solution using the same schedule as for the home animals.

Drug pretreatment. After the 5 days of habituation, animals in both the home and novel groups were assigned to one of six different subgroups, which received one of five doses of amphetamine (0.125, 0.5, 1.0, 4.0 or 8.0 mg/kg) or saline (0.0 mg/kg) for 5 consecutive daily infusions of the same dose. During this time animals in the home environment were treated in the same manner as during the habituation period, except the 40-μl portion of the infusion line closest to the catheter was filled with one of the 5 doses of amphetamine, or saline, and a tiny air bubble was placed between drug and saline to avoid diffusion of the drug into the saline. Again, after connecting the animals to the infusion line the experimenter left the room, and at either 1100, 1300 or 1500 hr the syringe pump was activated and delivered 80 μl at 20 μl/min over 4 min (20 μl catheter volume, 40 μl amphetamine/saline and 20 μl of heparin). The drug was infused at different times of the day so this could not serve as a cue predictive of drug administration. During the pretreatment phase animals in the novel group were transferred each day to a novel environment that was physically identical to the environment in which the animals in the home group were housed, their catheter was flushed with 0.1 ml of the heparin solution, and they were tethered and infused with amphetamine or saline in a manner identical to the home group.

Behavioral data were collected over a 2-hr period, after which animals in the novel group were returned to the main animal colony. Animals in the home group were left undisturbed until the end of the day, when the infusion line was removed. Rotational behavior was quantified by an automated program described previously (McFarlane *et al.,* 1992), with one rotation defined as four consecutive 90° turns in the same direction.

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Challenge test. After the pretreatment phase drug administration was discontinued for 5 days, during which time animals were handled in a manner identical to that described above for the habituation period, with one exception. The first day after the pretreatment phase all animals received an infusion of saline (saline challenge), to test for a conditioned response. Data on the saline challenge day was only collected for 1 hr, as the duration of the conditioned response is relatively short compared to the response to amphetamine.

On the sixth day after the last pretreatment infusion all animals received a challenge infusion of a fixed dose (0.5 mg/kg) of amphetamine to test for the expression of sensitization, and again rotational behavior was quantified for 2 hr.

Catheter Patency

To check for catheter patency at the end of the experiment, all animals received an i.v. infusion of 0.1 ml of sodium pentobarbital (50 mg/kg). The data from rats that did not become ataxic within 10 sec were excluded from analysis.

Data Analysis

Data are only presented for those animals that passed the apomorphine screening and catheter patency tests. The *N*s for each group are as follows: 0.0 mg/kg (home $N = 10$; novel $N = 10$), 0.125 mg/kg (home $N = 10$; novel $N = 8$), 0.5 mg/kg (home $N = 10$; novel $N = 10$), 1.0 mg/kg (home $N = 9$; novel $N = 9$), 4.0 mg/kg (home $N = 9$; novel $N = 9$) and 8.0 mg/kg (home $N = 11$; novel $N = 10$). Group differences in the total number of rotations averaged across the entire test session in response to the first injection of amphetamine were assessed using planned Student's *t* tests, and the time course of the drug effect was analyzed using two-way ANOVAs with repeated measures (test environment, i.v. home and i.v. novel and time). Within subjects comparisons (day 1 *vs.* 5) were made using two-way ANOVAs for repeated measures (day and dose) followed by planned paired *t* test comparisons. Between subjects comparisons for the amphetamine challenge and the saline challenge were made using two-way ANOVAs, followed by planned *t* tests.

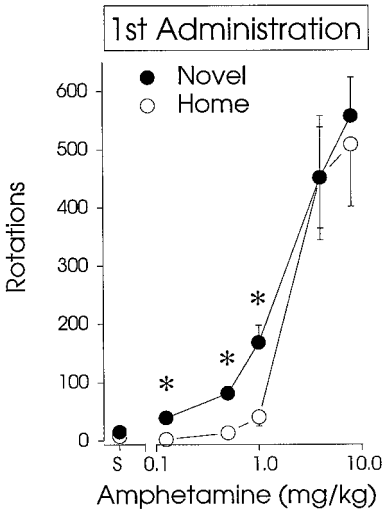

Fig. 1. The mean (±S.E.M.) number of rotations produced by the first intravenous infusion of five different doses of amphetamine or saline averaged over the 2-hr test session (where error bars are not evident they were smaller than the symbol). The open circles represent the home group and the closed circles represent the novel group. Planned *t* tests indicate that there were significant group differences (novel > home) for doses of 0.125 mg/kg ($t = -6.479$, $P < .0001$), 0.5 mg/kg ($t = -5.864$, $P < .0001$) and 1.0 mg/kg ($t = -3.935$, $P = .0012$).

Results

Effects of acute amphetamine. Figure 1 shows the number of rotations averaged over the entire test session following the first infusion, as a function of dose and environmental condition. With increasing dose there was an increase in the number of rotations produced by amphetamine in both groups. Lower doses (0.125–1.0 mg/kg) produced significantly more rotations in the novel group than in the home group, but there were not group differences for the two highest doses tested (4.0 and 8.0 mg/kg) (see fig. 1 legend for statistics). Figure 2 illustrates the time course of the behavioral response to the first amphetamine treatment and shows that the enhanced response seen in the novel group was due primarily to an increase in the magnitude of the response. Indeed, the same dose-effect relations were obtained if only the initial drug response (first 5 min) was considered (data not shown).

Effects of repeated amphetamine. One index of sensitization is provided by a within-subjects comparison of the

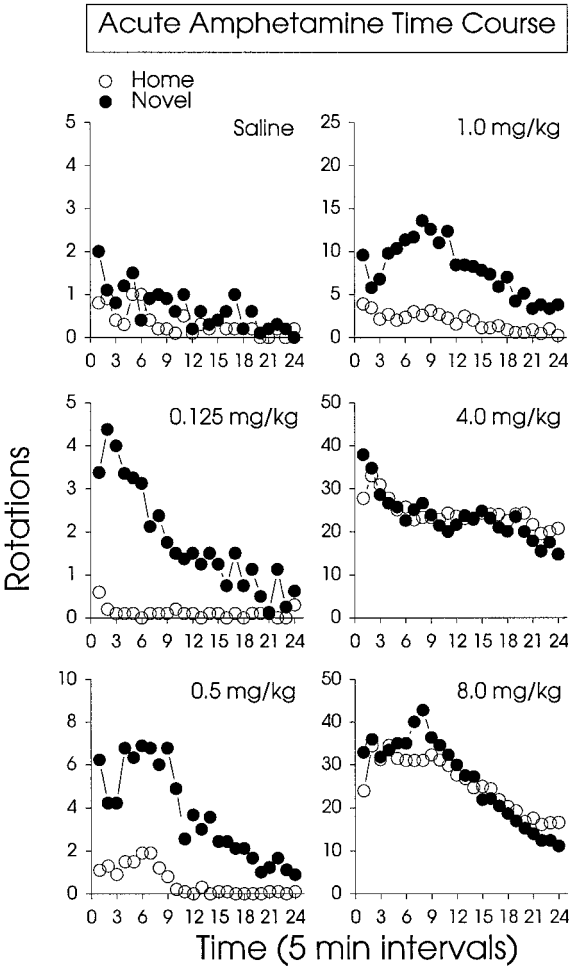

Fig. 2. The mean number of rotations per 5-min interval produced by the first infusion of amphetamine (0.125–8.0 mg/kg) or saline (0.0 mg/kg) for animals in both the home and novel groups (each panel represents a different dose, and note different scales). Animals in the home group are represented by open circles, and animals in the novel group are depicted by closed circles. There were significant group differences for doses between 0.125 and 1.0 mg/kg, inclusive (all P values < .001 for main effect of group and group by time interactions, two-way ANOVA), but no group differences for doses of 0.0, 4.0 or 8.0 mg/kg.

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psychomotor response on the first day of drug treatment with that on the last day of drug treatment (*i.e.,* a comparison between day 1 *vs.* 5). The results of this analysis are shown in figure 3. The top two panels (fig. 3, A and B) show data averaged across the entire 2-hr test session. When the data were analyzed over the entire test session the lowest dose to produce sensitization (day 5 more than 1) was 0.5 mg/kg for both the home and novel groups. The bottom two panels (fig. 3, C and D) provide an analysis of the initial drug effect (*i.e.,* the first 5-min interval after drug administration). This was done because the two major characteristics of sensitization are a more rapid onset of drug effect and an increase in the magnitude (*vs.* just duration) of drug effect (Leith and Kuczenski, 1982). Inspection of the time course of the drug effect indicates that both of these characteristics are captured by the initial drug response (*i.e.,* the first 5 min after infusion). The dose necessary to produce sensitization as indicated by

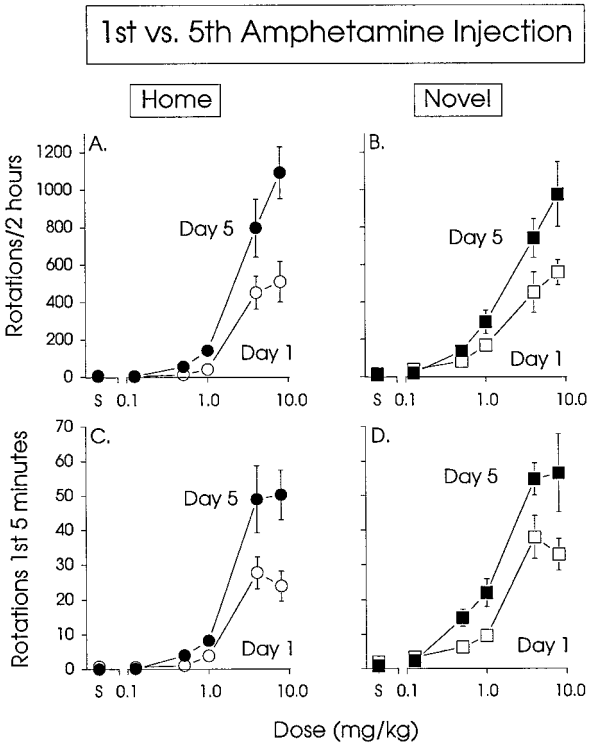

Fig. 3. The response (mean number of rotations, ±S.E.M.) to the first (open circles) *vs.* the fifth (closed circles) infusion of saline or different doses of amphetamine. A and B, Rotational behavior averaged over the entire 2-hr test session for the home and novel groups, respectively. For the home group an ANOVA resulted in a significant effect of day ($F = 15.267$, $P = .0002$), a significant effect of dose ($F = 44.452$, $P < .0001$) and a significant day-by-dose interaction ($F = 5.316$, $P = .0002$). For the novel group an ANOVA yielded a significant effect of day ($F = 11.716$, $P = .0009$), a significant effect of dose ($F = 40.237$, $P < .0001$) and a significant day by dose interaction ($F = 3.144$, $P = .0114$). Paired comparisons indicate that for both groups a dose of 0.5 mg/kg was the lowest dose to produce a significant increase in the response on day 5 compared to the response on day 1 ($P \leq .05$). C and D show the initial drug response, *i.e.,* the average of the first 5-min interval after drug administration for animals in the home group (C) and novel group (D). For the home group there was a significant effect of day ($F = 14.820$, $P = .0002$), a significant effect of dose ($F = 42.458$, $P < .0001$) and a significant day by dose interaction ($F = 4.47$, $P = .0010$). For the novel group there was also a significant effect of day ($F = 14.509$, $P = .0002$), a significant effect of dose ($F = 43.962$, $P < .0001$) and a significant day by dose interaction ($F = 2.635$, $P = .0281$). For the novel group a dose of 0.5 mg/kg was the lowest dose to produce sensitization ($P < .002$), whereas for the home group a dose of 1.0 mg/kg was required ($P = .0145$).

an increased initial drug effect differed for the two groups. For the novel group 0.5 mg/kg was required and for the home group 1.0 mg/kg was required.

Challenge test. A second index of sensitization is to compare the response of saline and drug-pretreated animals to a challenge infusion of a fixed dose. With a between-groups analysis, sensitization is indicated if drug-pretreated animals show a significantly greater psychomotor response to the drug challenge than saline-pretreated animals. In addition, in our experiment animals were pretreated with different doses of amphetamine, and therefore, it is possible to construct a dose-effect curve for the induction of sensitization. That is, it is possible to determine what pretreatment dose of amphetamine is required to induce sensitization.

Figure 4 shows the rotational behavior induced by a challenge infusion of 0.5 mg/kg of amphetamine, as a function of pretreatment dose and environmental condition. Figure 4, A and B show data averaged across the entire 2-hr test session and C and D show an analysis of the initial drug response (*i.e.,* the first 5-min interval after drug administration). It is clear from inspection of Figure 4A that there was a large effect of environment on the induction of sensitization, such that the dose-effect curve for the induction of sensitization in the novel group was shifted to the left and upward, relative to the home group. A direct comparison of the effect of pretreatment dose and group on the induction of sensitization was complicated, however, by the large group difference in the acute response to amphetamine (*i.e.,* between the groups pretreated with saline). Sensitization is indicated by the difference between the response of drug-pretreated and saline-pretreated animals, and this difference is better illustrated in Figure 4, B and D where the mean number of rotations for the respective saline-pretreated groups was subtracted from the scores for each animal in the appropriate amphetamine pretreated groups. Figure 4B shows that even after controlling for the group difference in the acute response to amphetamine there were still significant group differences in the dose-effect curves for the induction of sensitization. For the novel group the lowest pretreatment dose to produce sensitization (*i.e.,* a significantly greater response than saline pretreated animals) was 0.5 mg/kg. For the home group a pretreatment dose of 1.0 mg/kg was required to induce sensitization.

Inspection of figure 4, A and B suggests that in addition to a shift in the dose-effect curve for the induction of sensitization there was also an increase in the maximum effect of amphetamine in the novel group. This depends, however, on whether the entire time course of the drug effect is considered, or just the initial drug effect. Figure 4, C and D show the same analysis as in A and B, but for the initial drug effect (*i.e.,* the first 5-min interval). In this case there were no group differences at the highest pretreatment doses (4.0 and 8.0 mg/kg), although there was a significant shift to the left in the dose-effect curve for the novel group. The dose necessary for sensitization of the initial drug response was 0.125 mg/kg for the novel group, whereas for the home group a dose of 1.0 mg/kg was required.

Figure 5 shows the entire time course of the behavioral response to the 0.5 mg/kg challenge, and illustrates that in both the home and novel groups sensitization was characterized by an increase in the magnitude of the psychomotor response.

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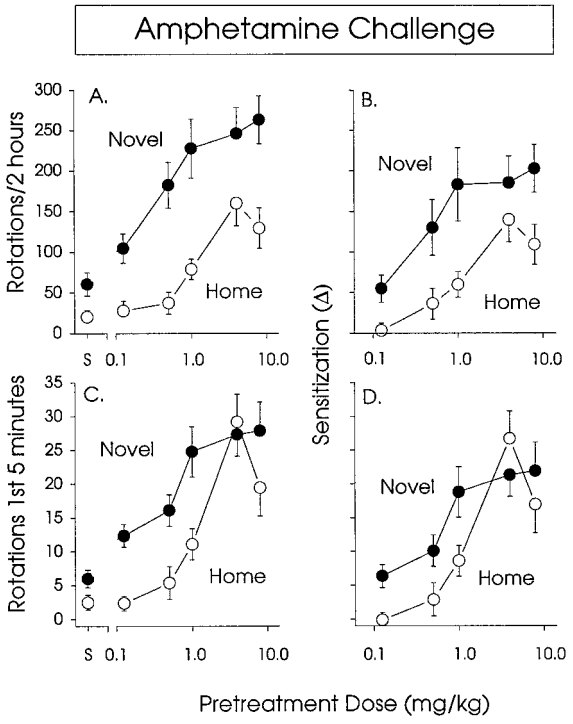

Fig. 4. The effects of a challenge infusion of 0.5 mg/kg of amphetamine on rotational behavior as a function of pretreatment condition. A, Illustrates the mean (±S.E.M.) number of rotations averaged across the entire 2-hr test session for animals in the home (open circles) and the novel (closed circles) groups. There were significant group differences (home *vs.* novel) in the effect of pretreatment condition on the response to an amphetamine challenge (a significant overall effect of environment ($F = 61.531$, $P < .0001$), a significant main effect of dose ($F = 17.139$, $P < .0001$) and an insignificant environment by dose interaction ($F = 1.836$, $P = .4687$). As expected (see fig. 1), however, there was also a significant effect of environmental condition on the saline-pretreated animals (novel > home), complicating a direct group comparison in A. This is because sensitization is defined as the difference in response between drug-pretreated and saline pretreated groups, but the saline pretreated groups differ. To "control" for this group difference in "baseline," therefore, B shows the same data as in A, but the average response in the appropriate saline-pretreated groups was subtracted from the score for each animal in the appropriate drug-pretreated groups, providing a difference score, which better illustrates the degree of sensitization in the home and novel groups. Even after controlling for group differences in the control response there was still significant effect of environmental condition on the dose-effect function of the induction of sensitization. A two-way ANOVA on the data in B yielded a significant overall effect of environment ($F = 24.145$, $P < .0001$), a significant main effect of dose ($F = 10.653$, $P < .0001$) and an insignificant environment by dose interaction ($F = 0.898$, $P = .4687$). Planned comparisons (t tests) were used to determine the lowest pretreatment dose necessary to induce sensitization (*i.e.,* a response significantly greater than in the saline pretreated group). For the home group the lowest dose to induce sensitization was 1.0 mg/kg ($P < .001$). For the novel group the lowest dose to induce sensitization was 0.5 mg/kg ($P < .001$). In the novel group there was a trend for sensitization following pretreatment with even 0.125 mg/kg, but this did not quite reach statistical significance ($P = .07$). C and D show the same analysis as for A and B, but for the initial drug response only (*i.e.,* the first 5 min after drug administration). A two-way ANOVA on the data in D yielded a significant overall effect of environment ($F = 5.388$, $P = .0227$), a significant main effect of dose ($F = 14.663$, $P < .0001$) and an insignificant environment by dose interaction ($F = 1.665$, $P = .1657$). Planned comparisons indicated that for the initial drug response a dose of 1.0 mg/kg was required to induce sensitization in the home group ($P < .002$), whereas for the novel group a dose of 0.125 mg/kg was sufficient ($P < .007$).

Saline challenge. To determine whether the drug administration procedure resulted in conditioned psychomotor activation, animals in both groups received an infusion of saline following the drug treatment phase. A two-way ANOVA was used to compare animals in the home condition with animals in the novel condition. There was a significant effect of environment ($F = 12.413$, $P = .0006$), but no effect of dose ($F = 1.519$, $P = .1903$) and no environment by dose interaction ($F = 1.525$, $P = .1884$). Only animals in the novel group treated with 0.5 mg/kg or higher showed a significant increase in rotations compared to saline pretreated animals (*i.e.,* a conditioned response) (for 0.5 mg/kg $t = -5.690$, $P < .0001$).

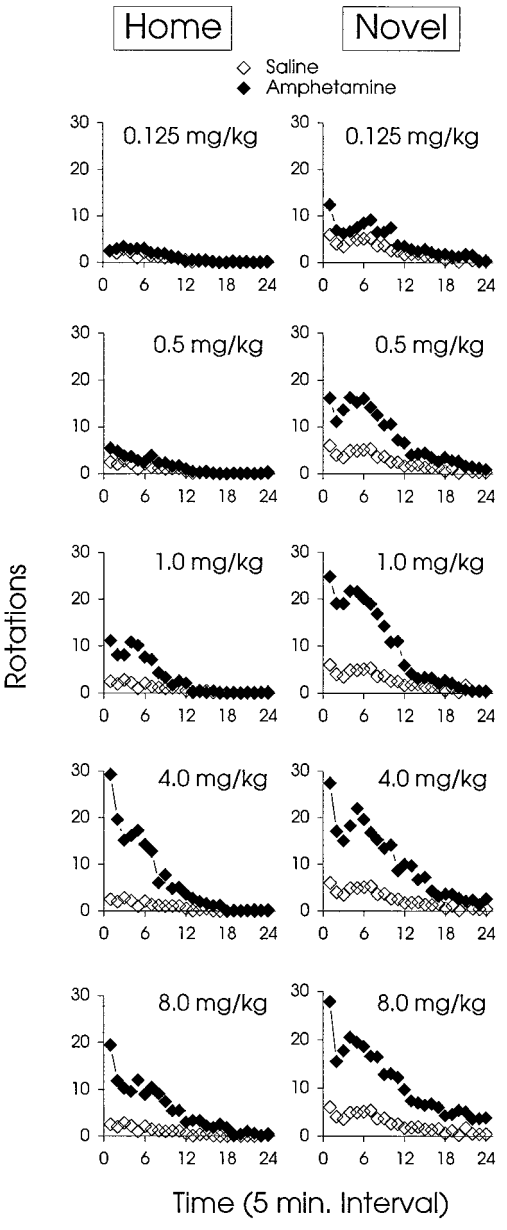

Fig. 5. The mean (±S.E.M.) number of rotations per 5-min interval over the 2-hr test session in response to a 0.5 mg/kg challenge in animals pretreated with either saline (dose 0.0 mg/kg) or 0.125, 0.5, 1.0, 4.0 or 8.0 mg/kg of amphetamine. The home group is represented by open circles, although the closed circles depict data from animals in the novel group.

Discussion

Our major purpose was to determine if it is possible to induce psychomotor sensitization if repeated i.v. infusions of amphetamine are given in the absence of any explicit envi-

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ronmental stimuli predictive of drug administration, such as transport, placement in a relatively novel test environment, the presence of an experimenter or handling. The results demonstrate that it was possible to induce sensitization under these conditions. Nevertheless, environmental condition (home *vs.* novel) did have a significant effect on the minimal dose of amphetamine required to induce sensitization. When amphetamine administration was paired with transport and placement into a relatively novel test cage (novel group), significantly lower doses of amphetamine were required to induce sensitization than when amphetamine was administered at home in the absence of any experimenter related stimuli (home group). With high doses, however, sensitization was induced regardless of environmental condition. Thus, the effect of environmental novelty was to reduce the dose necessary to induce psychomotor sensitization.

Effect of environment on the acute psychomotor response to amphetamine. We reported previously that the acute administration of moderate doses of amphetamine, given either i.p. or i.v., produce greater psychomotor activation (locomotor activity or rotational behavior) when given in a relatively novel environment than when given in a physically identical environment in which the animals live (*i.e.,* home) (Badiani *et al.,* 1995a, b; Crombag *et al.,* 1996). Our study confirms these observations, and further establishes that this effect of environment is limited to low or moderate doses. The highest doses tested (4.0–8.0 mg/kg) produced a comparable amount of rotational behavior in the home and novel groups. These data suggest, therefore, that the effect of environmental novelty on the acute response to amphetamine is to enhance sensitivity to low doses, but with no change in the maximal psychomotor response seen with high doses (also see Badiani *et al.,* 1997a). This effect of environment on acute drug responsiveness seems to be relatively specific to amphetamine, because this same environmental manipulation has no effect on the acute rotational response to cocaine, when cocaine is given either i.p. (Badiani *et al.,* 1995b) or i.v. (Browman *et al.,* 1998).

The neurobiological mechanism(s) by which environment modulates the acute response to amphetamine is not known. It does not appear to involve alterations in amphetamine pharmacokinetics, because the brain and plasma levels of amphetamine are the same in rats given i.p. amphetamine at home or in a novel environment (Badiani *et al.,* 1997a). It also does not appear to involve modulation of amphetamine's primary neuropharmacological effect, which is to enhance the synaptic concentrations of dopamine. There is no effect of environment (home *vs.* novel) on the ability of amphetamine to increase dopamine overflow in the caudate nucleus or the nucleus accumbens, as indicated by *in vivo* microdialysis (Browman *et al.,* 1995; Ostrander *et al.,* 1997). There is, however, a large effect of environment on the apparent postsynaptic consequences of amphetamine administration. Badiani and colleagues (1997b) have found that environmental novelty greatly enhances the ability of amphetamine to induce the expression of mRNA for the immediate early gene, *c-fos,* throughout the striatal complex. Thus, the relationship between environmental modulation of amphetamine-induced immediate early gene expression and its ability to modulate the acute and long-term (see below) behavioral consequences of amphetamine administration may yield new insights about the neurobiological mechanisms by which environmental factors modulate drug action.

Effect of environment on the induction of amphetamine sensitization. We reported previously that the circumstances surrounding drug administration also modulate the psychomotor sensitization produced by repeated treatment with either amphetamine or cocaine. When given i.p. to animals living in the test environment (*i.e.,* home) both amphetamine and cocaine induce sensitization, but significantly less robust sensitization than if animals receive the same treatments in a physically identical but novel test environment (Badiani *et al.,* 1995a, b, c). Furthermore, when the experimenter-related stimuli that inevitably accompany i.p. administrations were removed by the use of i.v. infusions at home, low to moderate doses of amphetamine or cocaine failed to induce sensitization (Browman *et al.,* 1998; Crombag *et al.,* 1996; Robinson *et al.,* 1998). Our study confirms these observations, and further establishes that for amphetamine this effect of environment is dose dependent. High doses of amphetamine-induced sensitization regardless of environmental condition. We recently reported that the same is true for the psychomotor sensitization induced by cocaine (Browman *et al.,* 1998).

Analysis of the entire time course of the drug effect (see fig. 4, A and B) suggests that this environmental manipulation might not only have shifted the dose-effect curve for the induction of sensitization, but altered the maximal effect. That is, even the highest doses tested (4–8 mg/kg, i.v.) appeared to produce less robust sensitization when given at home than when given in a novel environment (these doses do represent maximal effect for this behavior because higher doses could not be used, as they were toxic in some animals). If manipulation of the circumstances surrounding drug administration could modulate the ability of amphetamine to induce sensitization in this manner (*i.e.,* alter maximum effect), this would suggest that environmental factors could set absolute limits on the magnitude of the neuroadaptive processes underlying sensitization.

This conclusion is tempered, however, by analysis of the initial drug response, as shown in figure 4, C and D. There did not appear to be any influence of environment on the initial drug response at the highest doses tested. That is, the highest doses tested produced comparable sensitization in the home and novel groups, suggesting that the effect of environment was only to shift the dose-effect curve, and not to alter maximum effect. This raises interesting questions regarding how behavioral sensitization is best quantified, questions to which there are probably not definitive answers.

For example, it has been suggested that two hallmarks of behavioral sensitization are 1) a more rapid onset of the drug response and 2) an increase in the magnitude of the behavioral effect (Leith and Kuczenski, 1982). In our study inspection of the time course of the drug response indicates that analysis of the first 5-min interval best captures these two features of the behavioral response. With i.v. administration the largest behavioral response is seen during the first 5 min after drug administration, especially after sensitization (fig. 5). Thus, the analysis shown in figure 4, C and D indicates that the environmental manipulation used did not set any absolute limit on the ability of amphetamine to induce the neurobiological adaptations responsible for behavioral sensi-

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tization. This conclusion is consistent with our studies using cocaine rather than amphetamine (Browman *et al.,* 1998).

The apparent shift in maximal effect seen when the entire time course of the drug response was considered appears to be due, therefore, to an effect on the duration of the drug response. Some researchers have reported that sensitization is sometimes characterized by an increase in the duration of a drug response (Antelman, 1988), but others have argued that this is not a necessary characteristic (Leith and Kuczenski, 1982). It is certainly the case that different behavioral components of the psychomotor response, occurring at different times after drug administration, can be dissociated (Leith and Kuczenski, 1982). Thus, the significance of this finding for understanding how environmental factors modulate the induction of sensitization will probably have to wait until we better understand the phenomenon.

Another dissociation between different indices of behavioral sensitization was seen in comparing the within- *vs.* between-subjects analysis of the data averaged over the entire test session. For the within-subjects analysis (day 1 *vs.* 5) there was no difference in the dose required to induce sensitization in the home and novel groups, but on the amphetamine challenge day (between-subjects) there was a significant effect of environment on the minimal dose required to induce sensitization (although note that for the initial drug response, first 5-min interval, there was a significant effect of environment for both indices). Similar dissociations between within- and between-subjects estimates of sensitization have been reported before. The reason for this difference is not clear, but a couple of points are relevant. First, a between-subjects analysis provides a more "conservative" index of sensitization, as this approach controls for potential changes in behavior due to repeated handling and other nonspecific variables associated with the treatment protocol. These factors would be present in both the drug and saline-pretreated animals, so would theoretically be accounted for by this comparison. Second, the challenge test involved a withdrawal period of 5 days. The expression of psychomotor sensitization is often time-dependent (Antelman, 1988) and the neuroadaptations seen early after the cessation of drug treatment may differ from those seen later (Kalivas and Stewart, 1991; Paulson and Robinson, 1995; White and Wolf, 1991). It is possible, therefore, that environmental stimuli are especially effective in modulating the more persistent neuroplastic adaptations involved in sensitization.

The mechanism(s) by which the environmental manipulations used influence the induction of sensitization is not known. We have previously suggested that the mechanism may be related to differences in the availability of stimuli predictive of drug administration (Browman *et al.,* 1998; Crombag *et al.,* 1996). The fact that a conditioned response was seen only in the novel group is consistent with the notion that transport and associated stimuli might increase the responsiveness of animals in the novel group, relative to animals in the home condition, and thus contribute to the expression of sensitization. However, animals in the home group pretreated with doses of 1.0 mg/kg or higher showed no evidence of a conditioned response, but did express sensitization on the challenge day. Furthermore, it was recently reported that presenting a discrete cue predictive of drug administration has only a small effect on the induction of sensitization in the home condition (Crombag *et al.,* 1997). These results suggest that drug predictability plays a minor role in the effect of environmental novelty on sensitization, and that the development of a conditioned response is not necessary for the induction of sensitization.

Alternatively, it is possible that the differences observed between the home and novel groups might be due to the action of the novel environment as a stressor. It is known that exposure to a novel environment produces neuroendocrine and neural changes indicative of stress (Friedman and Ader, 1967), and it is possible that this may facilitate the induction of sensitization in the novel group. However, it has been reported that adrenalectomy does not block the effect of environmental novelty on amphetamine sensitization, suggesting that adrenal hormones are not involved (Badiani *et al.,* 1995c). Nevertheless, other stress-related hormones such as corticotropin-releasing hormone could be involved (Cador *et al.,* 1993). Finally, the ability of environmental novelty to facilitate amphetamine induction of the immediate early gene, *c-fos,* as discussed above (Badiani *et al.,* 1997b), could also provide a mechanism by which the circumstances surrounding drug administration modulates the long-term neurobiological consequences of repeated amphetamine administrations.

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References

Antelman S (1988) Time-dependent sensitization as the cornerstone for a new approach to pharmacotherapy: Drugs as foreign/stressful stimuli. *Drug Dev Res* **14:**1–30.

Badiani A, Anagnostaras SG and Robinson TE (1995a) The development of sensitization to the psychomotor stimulant effects of amphetamine is enhanced in a novel environment. *Psychopharmacology* **117:**443–452.

Badiani A, Browman KE and Robinson TE (1995b) Influence of novel versus home environments on sensitization to the psychomotor stimulant effects of cocaine and amphetamine. *Brain Res* **674:**291–298.

Badiani A, Morano MI, Akil H and Robinson TE (1995c) Circulating adrenal hormones are not necessary for the development of sensitization to the psychomotor activating effects of amphetamine. *Brain Res* **673:**13–24.

Badiani A, Camp D and Robinson T (1997a) Enduring enhancement of amphetamine sensitization by drug-associated environmental stimuli. *J Pharmacol Exp Ther* **282:**787–794.

Badiani A, Oates MM, Day HEW, Watson SJ, Akil H and Robinson TE (1997b) Amphetamine-induced expression of c-fos in the striatum is modulated by environmental stimuli. *Soc Neurosci Abstr* **23:**522.

Browman KE, Badiani A and Robinson TE (1998) The influence of environment on the induction of sensitization to the psychomotor activating effects of intravenous cocaine in rats is dose-dependent. *Psychopharmacology* **137:**90–98.

Browman KE, Lalley JJ, Badiani A and Robinson TE (1995) Striatal dopamine concentrations following an acute treatment with amphetamine in a novel versus home environment. *Soc Neurosci Abstr* **21:**2090.

Brown ZW, Amit Z and Weeks JR (1976) Simple flow-thru swivel for infusions into unrestrained animals. *Pharm Biochem Behav* **5:**363–365.

Cador M, Cole BJ, Koob GF, Stinus L and LeMoal M (1993) Central administration of corticotropin releasing factor induces long-term sensitization to D-amphetamine. *Brain Res* **606:**181–186.

Crombag HS, Badiani A and Robinson TE (1996) Signalled versus unsignalled intravenous amphetamine: large differences in the acute psychomotor response and sensitization. *Brain Res* **722:**227–231.

Crombag HS, Badiani A and Robinson TE (1997) The effects of drug-predictive cues on sensitization to the psychomotor stimulant effects of amphetamine. *Soc Neurosci Abstr* **23:**2404.

Friedman SB and Ader R (1967) Adrenocortical response to novelty and noxious stimulation. *Neuroendocrinology* **2:**209–212.

Kalivas PW and Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Rev* **16:**223–244.

Leith NJ and Kuczenski R (1982) Two dissociable components of behavioral sensitization following repeated amphetamine administration. *Psychopharmacology* **76:**310–315.

McFarlane DK, Martonyi BJ and Robinson TE (1992) An inexpensive automated system for the measurement of rotational behavior in small animals. *Behav Res Meth Inst Comput* **24:**414–419.

Ostrander MM, Fraioli S, Browman KE, Badiani A and Robinson TE (1997) The effect of environment on the psychomotor response to amphetamine is not mediated by dopamine overflow in the shell of the nucleus accumbens. *Soc Neurosci Abstr* **23:**1090.

Paulson PE and Robinson TE (1995) Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. *Synapse* **19:**56–65.

Paxinos G and Watson C (1997) *The Rat Brain in Stereotaxic Coordinates, 3rd ed.* Raven Press, New York.
Robinson TE (1984) Behavioral sensitization: Characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. *Psychopharmacology* **84:**466–475.
Robinson TE and Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Res Rev* **11:**157–198.
Robinson TE, Browman KE, Crombag HS and Badiani A (1998) Modulation of the induction or expression of psychostimulant sensitization by the circumstances surrounding drug administration. *Neurosci Biobehav Rev* **22:**347–354.
Stewart J and Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol* **4:**289–312.
Weeks JR (1972) Long-term intravenous infusions, in *Methods in Psychobiology* (Meyers RD ed) pp 155–168, Academic Press, London.
White FJ and Wolf ME (1991) Psychomotor stimulants, in *The Biological Bases of Drug Tolerance and Dependence* (Pratt J ed) pp 153–197, Academic Press, New York.

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