

Amphetamine-induced rapid-onset sensitization: Role of novelty, conditioning and behavioral parameters

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Abstract

Background: Environmental factors may modulate sensitization to the locomotor-activating effects of psychostimulants. In addition, some parameters of locomotor activity seem to be more sensitive to detect cocaine-induced behavioral sensitization. We examined how novelty and conditioning can modulate a previously described rapid-onset type of behavioral sensitization to amphetamine (AMP) in mice, using total, peripheral and central open-field locomotion frequencies as experimental parameters.

Methods: In the first experiment, mice received an ip injection of saline (SAL) or 5.0 mg/kg AMP paired or not with the open-field or in their home-cages. Four hours later, all the animals received an ip SAL challenge injection and, 15 min later, were observed in the open-field for quantification of total, peripheral and central locomotion frequencies. The second experiment had a similar protocol, except that mice received a challenge injection of 1.5 mg/kg AMP.

Results: The priming AMP injection significantly increased all the parameters of locomotion of SAL-challenged mice firstly exposed to or previously paired (but not unpaired) with the open-field. AMP priming injection enhanced total and peripheral locomotion of all AMP-challenged mice but only increased central locomotion of mice submitted to novelty or environmental conditioning.

Conclusion: Our results showed: 1) the development of an AMP-induced rapid-onset sensitization to novelty and rapid-onset environmental conditioning in mice, 2) the potentiation of the AMP-induced rapid-onset sensitization to an AMP challenge injection by novelty and environmental conditioning and 3) the importance of measuring different locomotor activity parameters in behavioral sensitization experiments. © 2006 Elsevier Inc. All rights reserved.

Keywords: Novelty; Environmental conditioning; Amphetamine; Rapid-onset behavioral sensitization; Locomotion parameters; Mice

1. Introduction

A progressive and enduring increase in the psychomotor and positive reinforcing effects of amphetamine (AMP) and other drugs of abuse can be observed after repeated administration (Masur et al., 1986; Robinson and Becker, 1986; Piazza et al.,

1990; Wise et al., 1996; De Vries et al., 1998). This phenomenon, called behavioral sensitization, has received increasing attention because sensitization-related neuroplasticity in brain reward systems, specially in the mesoaccumbens dopamine system, may contribute to addiction (Kalivas and Stewart, 1991; Hooks et al., 1993; Robinson and Berridge, 1993). In rodents, behavioral sensitization is usually measured in terms of locomotion or stereotypy (Robinson and Becker, 1986; Wise et al., 1996; Camarini et al., 2000; Quadros et al., 2003).

There are several factors that may alter the rewarding effects of drugs of abuse, and, therefore, their behavioral sensitization.

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One important factor is the environmental cues surrounding drug administration. Indeed, although sensitization to the locomotor stimulant effects of psychostimulants and other drugs of abuse has been observed when drug injections are not paired with the observation environment (Bellot et al., 1996, 1997; Costa et al., 2001; Araujo et al., 2005; Chinen et al., 2006), under some experimental designs, the behavioral sensitization depends critically on the pairing of the drug locomotor stimulant effect with the observation environment (Jackson and Nutt, 1993; Carey and Gui, 1998a,b; Frussa-Filho et al., 2004). This environmental modulation is particularly interesting because it's well known that environmental cues trigger craving and drug-seeking behavior in humans (Childress et al., 1986; Niaura et al., 1988; Carter and Tiffany, 1999). Likewise, in rodents, a drug-free exposure to an environment previously paired with a psychostimulant induces an increase in their locomotor activity (Beninger and Hahn, 1983; Gold et al., 1988; Stewart and Vezina, 1991; Hotsenpiller et al., 2002; Hotsenpiller and Wolf, 2002; Chinen et al., 2006).

Another important factor that can influence behavioral sensitization to the locomotor stimulant effects of drugs of abuse is the exposure to a novel environment. Within this context, the response to novelty in animals has been suggested as an analog of the personality trait associated with increased risk for drug abuse (Rebec et al., 1997) and catecholaminergic neurons seem to play a role in the rewarding properties of novel stimuli (Piazza et al., 1989; Pierce et al., 1990; Weissenborn and Winn, 1992). Indeed, rats given free-choice access to a novel and a familiar environment, presented an increase in catecholaminergic activity in the medial prefrontal cortex and nucleus accumbens shell (areas related to the reinforcing properties of drugs of abuse) when they entered into the novel environment, but not into the familiar environment (Rebec et al., 1997).

These data suggest that novelty and the rewarding effects of drugs of abuse may share similar neuronal substrates and that novelty could possibly mimic the effects of some drugs of abuse, therefore playing an important role in the development and expression of behavioral sensitization. The importance of novelty in modulating behavioral sensitization has also been extensively studied by Badiani and colleagues (1995a,b,c, 1998), who have demonstrated that sensitization to the psychomotor activating effects of amphetamine and cocaine is enhanced when the animals receive the drug treatment in a novel environment (i.e., an environment different from their home-cages) rather than in their home-cages.

The extent to which behavioral sensitization is induced by a drug is also highly dependent on the nature of the pre-treatment regimen. In this respect, it has been demonstrated that it is not necessary to repeatedly administer AMP for long periods of time to produce behavioral sensitization. Indeed, a single injection of AMP has been reported to enhance both stereotypy (Browne and Segal, 1977; Ellison and Morris, 1981) and locomotor stimulation (Vanderschuren et al., 1999) produced by a subsequent injection of AMP given weeks later. Within this context, Kuczenski and Segal (1999a,b) demonstrated that intense and focused stereotyped behavior could be elicited in rats by a low, nonstereotypy dose of AMP

(0.5–1.5 mg/kg) given after only some hours (3–5 h) of a “priming” injection of 4.0 mg/kg AMP. Importantly, this rapid-onset type of behavioral sensitization to stereotyped behavior occurred in the absence of an altered caudate–putamen extracellular dopamine response. Likewise, we recently demonstrated in our laboratory (Chinen et al., 2006) the development of this rapid-onset sensitization phenomenon not only to the stereotyped behavior but also to the locomotor stimulant effects of AMP in mice. Moreover, we verified the development of a rapid-onset environmental conditioning, which can potentiate rapid-onset sensitization, although this phenomenon can be manifested in the absence of this conditioning.

Other interesting, but not less important, factors that should be considered when analysing the modulation of behavioral sensitization to psychostimulants are the parameters of locomotor activity usually recorded in open-field tests: total, peripheral and central locomotion frequencies (Goto et al., 1993). For example, Carey and Gui (1997) demonstrated that entries in the center zone of the open-field seemed to be the most sensitive parameter to better differentiate cocaine anti-habituation effects from environmental conditioning, since it does not undergo habituation in control animals like the other parameters of locomotor activity.

The aims of the present study were to 1) replicate the rapid-onset environmental conditioning observed in our earlier report (Chinen et al., 2006); 2) verify whether a priming injection of AMP can induce a rapid-onset sensitization to novelty in mice and 3) analyze how the rapid-onset sensitization to AMP, novelty and rapid-onset environmental conditioning can differentially affect the 3 parameters of the locomotor activity (total, peripheral and central locomotor activity) in mice.

2. Materials and methods

2.1. Subjects

Three-month-old Swiss EPM-M1 female mice of our own colony were housed under conditions of controlled temperature (22–23 °C) and lighting (12/12 h light/dark, lights on at 06:45 h). The animals were housed in polypropylene cages (32 cm × 42 cm × 18 cm), 15 per cage. Food and water were available ad libitum throughout the experiments. Animals used in this study were maintained in accordance with the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication N° 85–23, revised 1985).

2.2. Drugs

Amphetamine (Sigma) was freshly diluted in saline solution and was given intraperitoneally in the volume of 10 ml/kg body weight. Saline was used as control solution.

2.3. Open-field test

Fifteen minutes after injection, the animals were individually placed in the center of the open-field arena for direct quantification of locomotion frequency during 5 min. The

open-field apparatus used in the present study was a circular wooden box (40 cm in diameter and 50 cm high) with an open top and floor divided into 19 squares. Hand-operated counters were used to score the following behavioral parameters during 5 min:

- Total ambulation frequency = number of any floor unit entered;
- Peripheral square ambulation frequency = number of entrances into the floor units close to the walls of the apparatus;
- Central square ambulation frequency = number of entrances into any floor unit not close to the walls of the apparatus.

This period of time has been demonstrated to be effective in detecting amphetamine-induced behavioral sensitization in mice (Bellot et al., 1997; Costa et al., 2001; Frussa-Filho et al., 2004; Chinen et al., 2006). In addition, this short period of open-field observation session seems to be more suitable to investigate the effects of novelty on the rapid-onset behavioral sensitization phenomenon (i.e., a longer session could lead to habituation, masking the eventual effects of novelty on mice's behavior). The observer was always unaware of the experimental design. The animals were used only once.

3. Experimental procedure

Experiment 1. Rapid-onset behavioral sensitization to novelty and rapid-onset environmental conditioning induced by a priming amphetamine injection in mice.

Table 1
Design of experiments 1 and 2

Experiment 1									
Group	Treatment regimen								
NEXP	AMP 5 or	4h			SAL	15 min	OFQ		
NPAIR	SAL								
	OFE	AMP5 or	4h		SAL	15 min	OFQ		
PAIR	SAL								
	AMP 5 or	OFE	90 min		SAL	15 min	OFQ		
		4h							
Experiment 2									
Group	Treatment regimen								
NEXP	AMP 5 or	4h			AMP 1.5	15 min	OFQ		
NPAIR	SAL								
	OFE	AMP5 or	4h		AMP 1.5	15 min	OFQ		
PAIR	SAL								
	AMP 5 or	OFE	90 min		AMP 1.5	15 min	OFQ		
		4h							

AMP 5–5.0 mg/kg amphetamine ip injection; SAL — saline ip injection; AMP 1.5–1.5 mg/kg amphetamine ip injection; OFE — open-field exposure for 150 min; OFQ — open-field quantification for 5 min.

Sixty female Swiss mice were randomly allocated to 6 groups of 10 animals each: SAL-NEXP, AMP-NEXP, SAL-NPAIR, AMP-NPAIR, SAL-PAIR and AMP-PAIR. Animals were habituated for 150 min in an open-field immediately before (-NPAIR groups) or after (-PAIR groups) they received an intraperitoneal (ip) priming injection of saline (SAL) or 5.0 mg/kg amphetamine (AMP). Mice from the -NEXP groups received this injection in their home-cages. Four hours after their respective priming injections, all the animals received a SAL challenge injection and, 15 min later, were placed in the open-field for locomotor activity quantification (see Table 1 for more details).

Experiment 2. Effects of environmental novelty and previous environmental conditioning on the expression of rapid-onset sensitization of AMP-induced locomotor stimulation.

The experiment had a protocol similar to that described in experiment 1, except that all the animals received a challenge injection of 1.5 mg/kg AMP instead of SAL (see Table 1 for more details).

3.1. Statistical analysis

Locomotion frequency among the several groups was compared by Analysis of variance (ANOVA) followed by Duncan's test. A *p* value less than 0.05 was considered as a statistically significant difference for all comparisons made.

4. Results

Experiment 1. Rapid-onset behavioral sensitization to novelty and rapid-onset environmental conditioning induced by a priming amphetamine injection in mice.

Fig. 1A, B and C shows that animals of the AMP-NEXP and AMP-PAIR groups presented significantly higher total, peripheral and central locomotion frequencies than that presented by all the other groups [$F(5, 54)=8.22, 5.70$ and 9.95 respectively, $p<0.001$]. In addition, the locomotion frequency of AMP-NPAIR group did not differ from that of SAL-NPAIR group. The same pattern of locomotor activity was verified for all the parameters of locomotion (total locomotion, peripheral locomotion and central locomotion).

Experiment 2. Effects of environmental novelty and previous environmental conditioning on the expression of rapid-onset sensitization of AMP-induced locomotor stimulation.

Fig. 2A and B shows that mice of the AMP-NEXP, AMP-NPAIR and AMP-PAIR groups presented significantly higher locomotion frequencies than that presented by all SAL groups in total [$F(5, 54)=9.05, p<0.001$] and peripheral [$F(5, 54)=7.45, p<0.001$] locomotion parameters. Regarding central locomotion, however, only AMP-NEXP and AMP-PAIR groups presented a significant increase in locomotion frequency when compared to SAL-NPAIR and SAL-PAIR groups [$F(5, 54)=3.72, p<0.01$]. Interestingly, the AMP-NEXP and AMP-PAIR groups also had higher locomotion

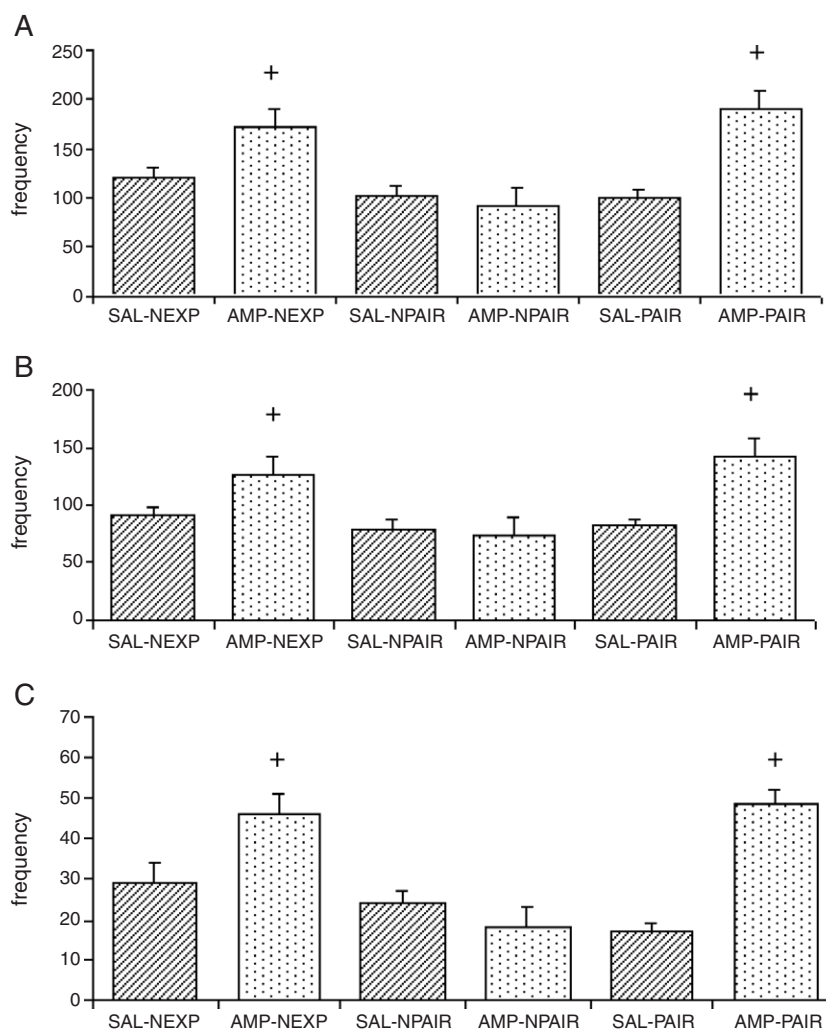


Fig. 1. Total locomotion frequency (A), peripheral locomotion frequency (B) and central locomotion frequency (C) of mice habituated for 150 min in an open-field immediately before (-NPAIR groups) or after (-PAIR groups) they received an intraperitoneal (ip) priming injection of saline (SAL) or 5.0 mg/kg amphetamine (AMP). Mice from the -NEXP groups received this injection in their home-cages. Four hours after their respective priming injections, all the animals received a SAL challenge injection and, 15 min later, were placed in the open-field for locomotor activity quantification. ⁺ $p < 0.001$ compared to SAL and AMP-NPAIR groups. ANOVA followed by Duncan's test.

frequencies than that presented by the AMP-NPAIR group [$F(5, 54) = 3.72$, $p < 0.01$].

5. Discussion

The major findings of the present study were that: 1) a priming injection of a high dose of AMP (5.0 mg/kg) was able to induce a rapid-onset sensitization to novelty – i.e., a novel environment – as well as a rapid-onset environmental conditioning. The latter replicates our finding in a previous report (Chinen et al., 2006); 2) the same pattern of these rapid-onset phenomena was observed for all the parameters of locomotor activity in mice; 3) environmental novelty and conditioning can similarly potentiate the rapid-onset sensitization to the locomotor-activating effects of AMP and 4) opposite to what happens on classical behavioral sensitization to the locomotor stimulant effects of cocaine (Carey and Gui, 1998a,b; Carey et al., 2005), central locomotion frequency is a less sensitive parameter to demonstrate the rapid-onset sensitization to AMP.

The first experiment of this study was aimed to replicate the rapid-onset environmental conditioning observed in our previous report and to verify whether a priming injection of 5.0 mg/kg AMP (dose used by Chinen et al., 2006 to demonstrate the rapid-onset sensitization phenomenon to AMP) could induce a rapid-onset sensitization to a novel stimulus, which may share similar anatomical substrates with drugs of abuse (Rebec et al., 1997). To test this hypothesis, all the animals previously treated with SAL or AMP received, at a 4-h interval, a challenge injection of SAL. Indeed, only the mice which had received a priming injection of AMP paired with the test environment (AMP-PAIR) or in their home-cages (AMP-NEXP) – and, therefore, exposed to novelty on the test session – presented an increase in locomotion frequencies of all the parameters recorded when compared to all the other groups. Concerning the conditioning data, they confirm previous experiments of our group (Chinen et al., 2006) and indicate that an environmental conditioning can be established very rapidly, suggesting that at least some neuroadaptations induced by drug–environment interactions may

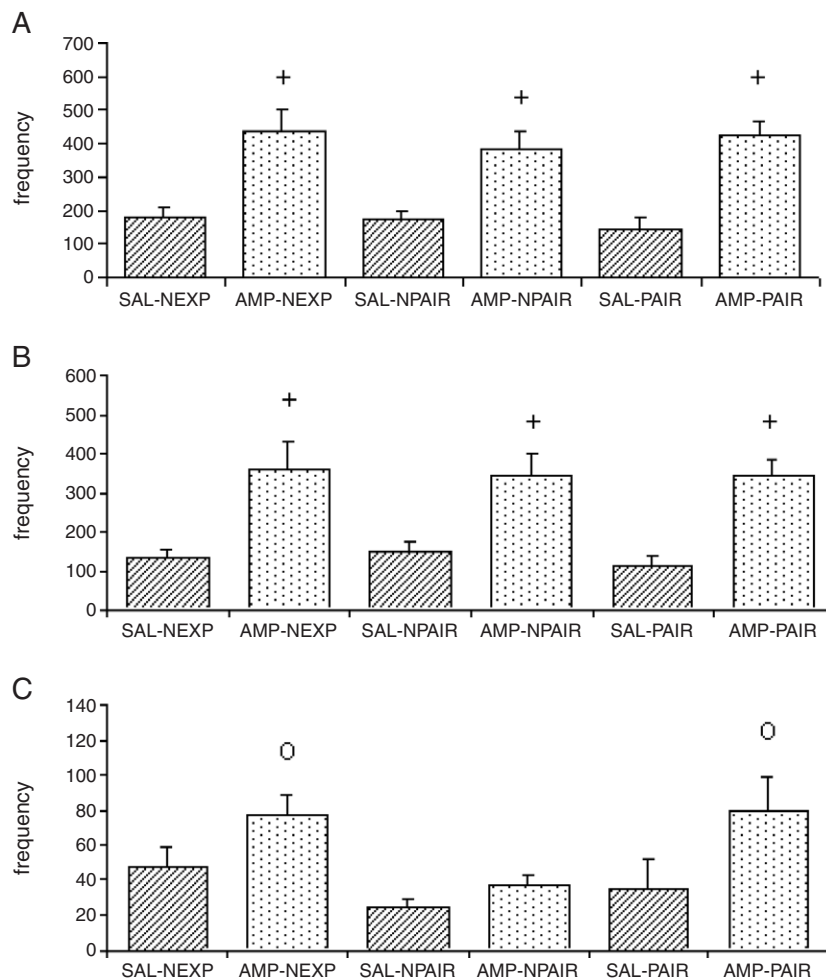


Fig. 2. Total locomotion frequency (A), peripheral locomotion frequency (B) and central locomotion frequency (C) of mice habituated for 150 min in an open-field immediately before (-NPAIR groups) or after (-PAIR groups) they received an intraperitoneal (ip) priming injection of saline (SAL) or 5.0 mg/kg amphetamine (AMP). Mice from the -NEXP groups received this injection in their home-cages. Four hours after their respective priming injections, all the animals received a challenge injection of 1.5 mg/kg AMP and, 15 min later, were placed in the open-field for locomotor activity quantification. ⁺ $p < 0.05$ compared to SAL groups. [°] $p < 0.05$ compared to animals of the SAL-NPAIR, SAL-PAIR and AMP-NPAIR groups. ANOVA followed by Duncan's test.

emerge in a very short period of time. Within this context, it is important to note that the present results extend the demonstration of this rapid-onset environmental conditioning to other behavioral parameters besides total locomotion: central and peripheral locomotion. As for the novelty data, they demonstrate, for the first time in the literature, that similarly to an amphetamine challenge injection (Chinen et al., 2006 and data from experiment 2), novelty can express behavioral sensitization after one single priming injection of this drug.

Interestingly, the magnitude of the rapid-onset sensitization to novelty was similar to that of environmental conditioning. This fact opens the possibility that the increase in the locomotor activity presented by animals of the AMP-PAIR group when compared to the SAL-PAIR group, after saline challenge, could be due to an amnesic effect of AMP instead of environmental conditioning of its stimulant effect. However, this possibility seems unlikely since the locomotor activity of the AMP-PAIR group was significantly higher than that of the SAL-NEXP group. In addition, under the present experimental conditions, the previous exposure to the open-field induced only a mild

decrease in the locomotor activity of saline-pretreated mice, which did not reach significance for any parameter (there were no significant differences between SAL-NEXP, SAL-NPAIR and SAL-PAIR groups after saline challenge).

From another standpoint, one could argue that the effects observed for novelty and conditioning could be due to residual levels of AMP or increased extracellular dopamine. Nevertheless, this does not seem to be the case, since we demonstrated in the first experiment that groups exposed to the open-field in the absence of the drug and immediately later treated with SAL (SAL-NPAIR) or AMP (AMP-NPAIR), did not differ from each other in terms of locomotor activity when they were challenged with SAL 4 h after these injections (Fig. 1A, B and C). Furthermore, Kuczenski and Segal (1999a) demonstrated that the rapid-onset (3-h interval) sensitization to AMP in rats occurred without a corresponding increase in extracellular dopamine levels. In this respect, after AMP administration, extracellular dopamine concentrations are highly correlated with extracellular concentrations of AMP, and the rate constants for the decline of extracellular dopamine, extracellular AMP,

and tissue levels of AMP are comparable (Kuczenski et al., 1997).

By another point of view, one could also argue that the absence of differences in locomotion frequencies observed for AMP-NPAIR and SAL-NPAIR groups challenged with SAL could be due to the development of a backward aversive conditioning in animals from the AMP-NPAIR group (i.e., a suppression of activity), which, in this case, would have learnt that being in the test environment predicted drug administration. Nevertheless, this possibility seems unlikely, since we have conducted an experiment comparing animals injected with AMP immediately after open-field exposure (as AMP-NPAIR groups from experiment 1) with animals injected with AMP 1 h after open-field exposure (to eliminate the possible backward conditioning) and there was no significant difference between the locomotion frequencies of these groups (data not shown). In further support to this idea, Cunningham et al. (1997) have demonstrated that administration of ethanol immediately after the CS (conditioned stimulus) exposure produced place aversion whereas administration of this drug 15 or 60 min after the CS exposure produced no place conditioning at all. In addition, the NPAIR groups of our study were exposed to the open-field for a very long time (150 min) before drug administration. In this regards, Cunningham and Henderson (2000) verified that longer conditioning trials (60 or 90 min) in the ethanol-induced place aversion paradigm produced only a non-significant trend toward place aversion. Together, all these evidence support the idea that the backward conditioning is not related to the absence of behavioral differences between AMP-NPAIR and SAL-NPAIR groups.

As far as we know, this is the first paper showing that a previous injection of AMP can sensitize mice to novelty. In fact, usually most of the studies involving novelty and behavioral sensitization in rodents focus on studying how different behaviors toward novelty can be correlated with behavioral responses to drugs of abuse, and, consequently, with propensity to addiction (Piazza and Le Moal, 1996; Chefer et al., 2003; Orsini et al., 2004). Despite all these studies, none has ever studied whether the exposure to a novel environment alone (i.e., without an amphetamine challenge injection) after a previous psychostimulant treatment can induce the expression of this phenomenon.

It's still not clear how novelty induces the expression of the rapid-onset sensitization to AMP. Our hypothesis is that novelty would be functioning as a positive reinforcer, and therefore, would act similarly to drugs of abuse, increasing extracellular dopamine in the mesolimbic system and inducing the expression of the rapid-onset sensitization phenomenon. Supporting this hypothesis, it's known that other reinforcers, like natural reinforcers, such as food and sex, also promote an increase in extracellular dopamine in the mesolimbic system of rodents (Hernandez and Hoebel, 1988a,b; Damsma et al., 1992; Pfau et al., 1995), similarly to cocaine (Hernandez and Hoebel, 1988a). Moreover, Rebec et al. (1997) demonstrated that entries into the novel environment increased catecholaminergic activity in brain areas of rats related to reinforcement. However, it's a general knowledge that stress can also increase extracellular

dopamine in structures of the mesolimbic system (Abercrombie et al., 1989) and that exposure to a novel environment may also act as a stressor, as mentioned earlier by Badiani et al. (1995c). Therefore, the activation of dopaminergic systems by novelty-induced stress could also contribute for the expression of the AMP-induced rapid-onset sensitization observed in our study. Despite all these possibilities, our data don't allow us to determine which one is more likely. Thus, this issue still remains to be explored.

Another important finding of the present investigation was that environmental novelty and conditioning may equally potentiate the rapid-onset sensitization to the locomotor stimulant effects of AMP, although this phenomenon can develop in the absence of both these factors (experiment 2). At a first glance one could argue that environmental novelty and conditioning did not modify the rapid-onset sensitization to AMP. Indeed, considering the total and peripheral locomotion parameters, all the animals pretreated with AMP presented a robust rapid-onset sensitization to the AMP challenge injection, which had the same magnitude irrespectively of their specific environmental history (Fig. 2A and B). However, central locomotion data revealed a clearcut potentiation effect of novelty and environmental conditioning on AMP-induced rapid-onset sensitization. In effect, when evaluated by the central locomotion parameter, the rapid-onset sensitization phenomenon was observed only in mice exposed to a novel environment or previously submitted to environmental conditioning. In addition, central locomotion frequencies of the AMP-NEXP and AMP-PAIR groups were significantly higher than that of the AMP-NPAIR group. Thus, the possibility is raised that, for the two other parameters of locomotor activity (total and peripheral), the magnitude of the rapid-onset sensitization to AMP was so high that it led to a ceiling effect, which, therefore, omitted the potentiation of this phenomenon by environmental novelty and conditioning. In order to confirm that the potentiation of the rapid-onset sensitization to AMP was in fact being masked by a ceiling effect, an additional experiment was performed in which we reduced the priming dose of AMP to 2.5 mg/kg and the challenge dose of AMP to 1.0 mg/kg and compared the three AMP-treated and -challenged mice (NEXP, NPAIR and PAIR — data not shown). As a result, we verified that the AMP-NEXP and the AMP-PAIR groups presented higher locomotion frequencies (total, peripheral and central locomotion frequencies) than the AMP-NPAIR group, demonstrating that our results from experiment 2 were really an outcome of the ceiling effect produced by the high doses of AMP utilized.

Our data from experiment 2 also suggest that, opposite to cocaine-induced classical behavioral sensitization (late onset, associated with repeated drug administration) in rats (Carey and Gui, 1998a,b; Carey et al., 2005), central locomotor activity seems to be a less sensitive parameter to measure AMP-induced rapid-onset sensitization in mice. This concern notwithstanding the lower sensitivity of this parameter was useful in our study, since it allowed the demonstration of the potentiation of rapid-onset sensitization to AMP by both environmental conditioning and novelty.

The potentiation of classical behavioral sensitization by novelty or environmental conditioning has already been

demonstrated. Concerning the novelty influence on traditional behavioral sensitization, Badiani and colleagues (1995a,b,c) in a series of reports, demonstrated that when AMP is administered in a novel environment, its acute psychomotor activating effects (rotational behavior in 6-OHDA lesioned rats and locomotor activity) and the degree of sensitization are greater than when this drug is administered in home-cages that are physically identical to the novel environment. As for the conditioning modulation of behavioral sensitization, Carey and Gui (1998a) verified that rats which received a repeated treatment with cocaine paired with an open-field exhibited an earlier onset of behavioral sensitization after a cocaine challenge injection as compared with animals previously treated with saline or cocaine unpaired with the open-field (exposed to the apparatus 30 min before drug administration). Likewise, Anagnostaras and Robinson (1996) demonstrated that rats given an AMP treatment paired with a rotometer presented greater sensitization (an increase in rotational behavior) after an AMP challenge injection than rats given the AMP treatment in an unpaired design (AMP after exposure to the rotometer). However, there are no studies comparing the magnitude of the effects of these two factors (novelty and environmental conditioning) on the expression of behavioral sensitization. We demonstrated here that novelty and environmental conditioning can potentiate the rapid-onset type of behavioral sensitization to AMP with a similar magnitude.

In summary, our results emphasize that environmental factors can induce dramatic and very rapid-onset effects on the behavioral consequences of a priming injection of amphetamine, including the behavioral sensitization phenomenon. We also demonstrated the importance of recording different parameters of locomotor activity in experiments involving behavioral sensitization.

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