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Abstract

Preclinical studies have shown that repeated stress experiences can result in an increase in the locomotor response to the subsequent administration of drugs of abuse, a phenomenon that has been termed behavioral cross-sensitization. Behavioral sensitization reflects neuroadaptive processes associated with drug addiction and drug-induced psychosis. Although cross-sensitization between stress- and drug-induced locomotor activity has been clearly demonstrated in adult rats, few studies have evaluated this phenomenon in adolescent rats. In the present study, we determined if the simultaneous exposure to stress and nicotine was capable of inducing behavioral sensitization to nicotine in adolescent and adult rats. To this end, adolescent (post-natal day (P) 28-37) and adult (P60-67) rats received nicotine (0.4 mg/kg, sc) or saline (0.9% NaCl, sc) and were immediately subjected to restraint stress for 2 h once a day for 7 days. The control group for stress was undisturbed following nicotine or saline injections. Three days after the last exposure to stress and nicotine, rats were challenged with a single dose of nicotine (0.4 mg/kg, sc) or saline and nicotine-induced locomotion was then recorded for 30 min. In adolescent rats, nicotine caused behavioral sensitization only in animals that were simultaneously exposed to stress, while in adult rats nicotine promoted sensitization independently of stress exposure. These findings demonstrate that adolescent rats are more vulnerable to the effects of stress on behavioral sensitization to nicotine than adult rats.

Key words: Cross-sensitization; Locomotor activity; Nicotine; Stress; Adolescence

Introduction

Drug abuse most commonly begins during adolescence, a period of ontogeny during which individuals exhibit age-specific behavioral characteristics, such as risk taking and novelty seeking, which could predispose them to initiate drug use (1,2). As observed for other drugs of abuse, it has been demonstrated that the initiation of tobacco smoking occurs during early adolescence (3). Many studies have shown that tobacco use during adolescence produces enduring effects that may increase the vulnerability to addiction later in life (4,5). Moreover, pre-clinical evidence shows that nicotine is more rewarding and less aversive during adolescence than during adulthood (6,7).

In animals, repeated administration of drugs of abuse may result in a gradual increase of the motor stimulant response with each subsequent injection. This phenomenon is termed behavioral sensitization, which is suggested to reflect neuroadaptive processes associated with drug addiction (8-10) and drug-induced psychosis (11). Despite the variability observed in the acute effects of nicotine on locomotor activity, repeated administration of this drug induces behavioral sensitization in adult rats (12-15). Although adolescent rats, as compared to adults, were more sensitive to the acute effect of nicotine on locomotor activity (8,16), they did not express behavioral sensitization to nicotine after repeated administration of this substance (13,14,17).

Exposure to stress appears to increase the number of cigarettes smoked and is strongly associated with craving and relapse of tobacco smoking (18,19). Preclinical studies

have shown that repeated stress experiences can result in an increase in the locomotor response to subsequent administration of drugs, a phenomenon that has been termed behavioral cross-sensitization (20-27). For instance, cross-sensitization between stress and psychostimulants has been observed after repeated exposure to footshock (28,29), restraint (30), food restriction (31), and social defeat stress (8,32).

The results regarding stress-promoting cross-sensitization to nicotine in adult rats are very contradictory. For example, Kita et al. (33) demonstrated that in adult rats repeated exposure to social stress enhanced nicotine-induced behavioral sensitization, whereas Faraday et al. (34) failed to observe changes in the locomotor response to nicotine administered with mini-pumps in rats exposed to restraint stress. Moreover, a study from our laboratory showed that previous exposure to restraint stress for 7 days did not affect nicotine-induced locomotor-activating effects in adult and adolescent rats (14).

Despite the strong influence of stress on tobacco addiction, the effects of simultaneous exposure to repeated stress and nicotine on nicotine-induced behavioral sensitization have not been investigated. Thus, the objective of the present study was to examine the effects of simultaneous exposure to stress and nicotine on nicotine-induced behavioral sensitization in adolescent and adult rats.

Subjects and Methods

Subjects

Male Wistar rats were obtained from the animal breeding facility of Universidade Estadual Paulista (UNESP, Botucatu, SP, Brazil) immediately after weaning, on postnatal day (P) 21. Animals were housed in 32-cm wide x 40-cm long x 16-cm high plastic cages (4 animals/cage) in a room maintained at 23 ± 2°C on a 12:12-h light/dark cycle (lights on at 7:00 am) and were allowed free access to food and water. All experiments were performed during the light phase. Each animal was used only in one experimental procedure. The experiments were carried out between 8:00 and 12:00 h.

Adolescence was defined, according to Spear (2), as the age period between P28 and P42. Experiments were started on P28 for adolescent rats and on P60 for adult rats.

The experimental protocol was approved by the Ethics Committee for Use of Human or Animal Subjects of Faculdade de Ciências Farmacêuticas, UNESP (CEP-24/2003) and the experiments were conducted according to the ethical principles of the Brazilian College of Animal Experimentation (COBEA), based on NIH Guidelines for the Care and Use of Laboratory Animals**.**

Drug

Nicotine® (Sigma, USA) was diluted in saline (0.9% NaCl), pH 7.0.

Apparatus

Behavioral testing was conducted in commercially available (Columbus Instruments, USA) activity-monitoring chambers consisting of 40-cm wide x 40-cm long and 16-cm high Plexiglas cages. The chambers contained 10 pairs of photocell beams, which were used to measure the horizontal locomotor activity. The consecutive interruption of two beams was recorded as one locomotion unit.

Nicotine- and stress-induced behavioral sensitization

The entire sensitization protocol took 10 days. On days 1 to 7, rats were weighed and given *sc* injections of nicotine (0.4 mg/kg) or saline once a day and immediately subjected to restraint stress for 2 h. The control animals were undisturbed in their home cages after the injections of nicotine or saline. On days 8 and 9 the animals did not receive any treatment.

On day 10 animals were allowed a 20-min adaptation period to the photocell apparatus and were then immediately injected with a challenge dose of saline (adolescents: non-stress, N = 13; chronic stress, N = 12; adults: non-stress, N = 14; chronic stress, N = 12) or nicotine (0.4 mg/kg, *sc*) (adolescents: non-stress, N = 14; chronic restraint, N = 13; adults: non-stress, N = 14; chronic restraint, N = 13). Immediately after the injections, the animals were returned to the activity chambers and their locomotor activity was recorded during a 30-min testing session as described above.

Adolescent and adult rats were on P37 and P69, respectively, when the behavioral tests were performed.

Statistical analysis

All data are reported as means ± SEM. Levine's tests for homogeneity of variance performed for the behavioral data showed no statistically significant differences, indicating homogeneity of variance. Thus, locomotor activity data for both adolescent and adult rats were analyzed by 2 x 2 x 2 ANOVA [drug treatment (nicotine *vs* saline) x stress (stress *vs* non-stress) x challenge (nicotine *vs* saline)]. When a significant ($P < 0.05$) main effect was observed, the Newman-Keuls test was used for *post hoc* comparisons.

Results

Adolescent rats

Three-way ANOVA showed significant differences regarding drug treatment [$F_{(1,44)} = 11.13$; $P < 0.005$], challenge [$F_{(1,44)} = 45.42$; $P < 0.001$], but not stress [$F_{(1,44)} = 0.08$; $P = 0.78$] factors. In addition, a significant interaction was detected between the three factors [$F_{(1,44)} = 5.85$; $P < 0.05$] (Figure 1).

Considering the control groups, further analysis (Newman-Keuls test) revealed that nicotine increased locomotor activity in rats pretreated with saline (SAL) or nicotine (NIC). However, no significant difference was observed between the SAL+NIC and NIC+NIC groups, indicating that pretreat-

ment with nicotine did not cause locomotor sensitization.

For the stress groups, further analysis (Newman-Keuls test) revealed that nicotine increased locomotor activity in rats pretreated with saline or nicotine. In addition, the locomotor response to a nicotine challenge was higher in the NIC+NIC group when compared to the SAL+NIC group, indicating that pretreatment with nicotine caused locomotor sensitization in animals simultaneously exposed to stress and nicotine.

Adult rats

Three-way ANOVA revealed significant differences in nicotine-induced locomotion considering treatment [$F_{(1,45)}$ = 33.95; $P < 0.001$] and challenge factors [$F_{(1,45)}$ = 50.57; $P < 0.001$] but not stress [$F_{(1,45)}$ = 0.36; $P = 0.55$]. Furthermore, a significant interaction between treatment and challenge factors was detected [$F_{(1,45)}$ = 22.12; $P < 0.001$] (Figure 2).

For both the control and stress groups, the Newman-Keuls test revealed that nicotine challenge significantly increased locomotor activity only in animals pretreated with nicotine ($P < 0.01$), showing that pretreatment with nicotine caused locomotor sensitization to nicotine independently of stress.

No significant difference in nicotine challenge effect was found between rats repeatedly exposed to restraint stress and the non-stress group ($P > 0.05$).

Discussion

In adolescent rats, we observed that nicotine caused behavioral sensitization only in animals that were simultaneously exposed to stress, while in adult rats nicotine promoted sensitization in both the stress and control groups. These data are the first to demonstrate the effects of repeated and simultaneous exposure to stress and nicotine on the locomotor response to this drug.

The observation that repeated nicotine administration did not induce locomotor sensitization in adolescent rats corroborates findings demonstrating that repeated treatment with nicotine (0.4 mg/kg) during adolescence did not induce behavioral sensitization (13,14,17). Moreover, data from our laboratory demonstrated that the previous exposure to stress was not able to change the locomotor response to a subsequent injection of nicotine in male adolescent rats (22). Additionally, McCormick and Ibrahim (35) showed that 1-h isolation had no effect on nicotine-induced locomotor activity in adolescent rats. However, in the present study, when adolescent rats received nicotine and were immediately exposed to stress, they showed a clear-cut sensitization of locomotor activity. Taken together, these results suggest that simultaneous exposure to nicotine and stress is necessary for the development of behavioral sensitization to this drug in adolescent rats.

Compared to studies that used previous exposure to
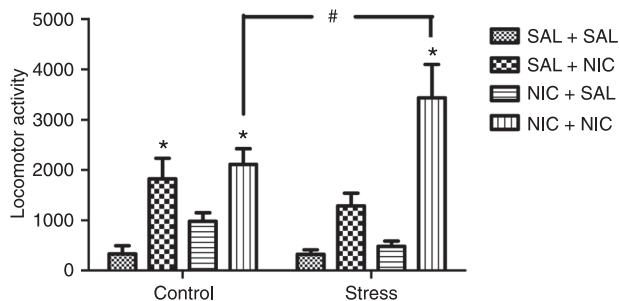
Figure 1. Effect of simultaneous exposure to stress and nicotine on locomotor activity in response to a nicotine injection in adolescent rats. SAL = 0.9% NaCl; NIC = nicotine. *$P < 0.05$ compared to all other groups; #$P < 0.05$ compared to NIC+NIC control group (Newman-Keuls test).
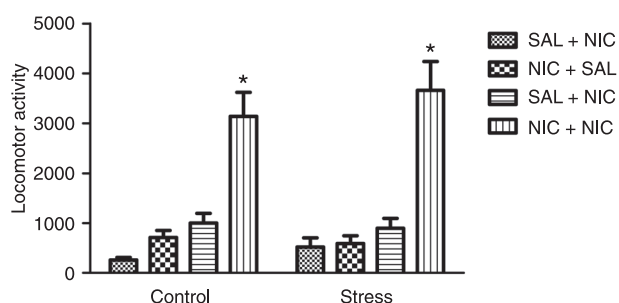
Figure 2. Effect of simultaneous exposure to stress and nicotine on locomotor activity in response to a nicotine injection in adult rats. SAL = 0.9% NaCl; NIC = nicotine. *$P < 0.05$, compared to all other groups (Newman-Keuls test).

stress to evaluate the cross-sensitization to nicotine (22,35), our experimental design (simultaneous exposure to stress and nicotine) appears to be more predictive of the human situation, in which stress and drug use are concomitant (18,19).

The repeated administration of nicotine to adult rats induced behavioral sensitization in both the stress and non-stress (control) groups. These findings also agree with other reports describing that repeated nicotine treatment causes behavioral sensitization in adult rats (12-14,36). Moreover, the fact that stress did not induce cross-sensitization to nicotine corroborates previous reports showing that pre-exposure to restraint stress did not change the effects of nicotine on locomotor activity (14,34). On the other hand, it was demonstrated that rats exposed to psychological stress for 10 days (by being exposed to the emotional responses of animals that received foot-shock) were sensitized to nicotine-induced ambulatory stimulation (33). Stress-induced cross-sensitization to nicotine in adult animals remains controversial and seems to depend on the

intensity, duration, frequency and timing of stress exposure. In our study, we also observed that stress did not increase the locomotor sensitization that was induced by repeated nicotine administration. This fact may have been due to a ceiling effect of nicotine-induced locomotor sensitization at the dose 0.4 mg/kg. Future studies using lower doses will be carried out in our laboratory.

Since animals were tested in a novel environment, we cannot rule out that stress-induced changes in the response to novelty may interfere with the measurement of the stimulant effects of nicotine. Previous data from our laboratory have shown that adult rats exposed to 14 days of repeated restraint stress showed an increased locomotor response to novelty and sensitization to cocaine-induced locomotor activity (21). However, in adolescent rats the exposure to both chronic restraint and variable stress increased cocaine-induced locomotion, but did not change the response to a novel environment (37).

These results suggest that stress-induced changes in the locomotor response to novelty are not related to the cross-sensitization between stress and drugs, at least in adolescent animals.

Since behavioral sensitization reflects neuroadaptive processes associated with drug addiction (9), our results suggest that simultaneous exposure to stress and nicotine might enhance the addictive properties of nicotine in adolescent rats.

Age-related differences in response to psychostimulants following exposure to stress have also been reported. For instance, it was demonstrated that restraint stress caused cross-sensitization to cocaine in adolescent, but not in adult rats (21,38).

Most available evidence suggests a critical role for the hypothalamus-pituitary-adrenal (HPA) axis in drug-induced sensitization (23,38). Concerning nicotine, Johnson et al. (39) showed that adrenalectomy disrupted the development, but not the expression, of sensitization, since it had no effect if performed in rats already sensitized. Taking these observations together, we could suppose that differences in the adaptation of HPA activity to stress between adult and adolescent rats could explain, at least in part, the results obtained in the present study. However, more investigations are necessary to characterize this relationship.

In rodents, these adaptations have been mainly monitored by comparing corticosterone levels in response to stress in adult and adolescent rats. For example, in adult rats when the same stressor is repeated the HPA response can desensitize or remain unchanged (for a review, see Ref. 40). Conversely, previous results from our laboratory have shown that exposure to repeated restraint stress during adolescence did not modify the HPA axis response to a new exposure to restraint stress (Cruz FC, Marin MT, Leão RM, Planeta CS, unpublished data), suggesting that adolescent rats are resistant to neuroendocrine adaptation to stressors compared to adult rats.

Overall, our results suggest that adolescents are more susceptible to stress influence on the development of nicotine addiction.

Acknowledgments

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