ELSEVIER

European Journal of Pharmacology 328 (1997) 103–114

ejp

Neonatal and preweanling rats are able to express short-term behavioral sensitization to cocaine

Ezio Tirelli *, Maurizio Ferrara

Biological Psychology and Pharmacopsychology Unit, University of Liège, Boulevard du Rectorat B-32, B-4000 Liège, Belgium

Received 27 March 1997; accepted 4 April 1997

Abstract

The present study assessed the ability of suckling rats to express short-term behavioral sensitization to cocaine prior to weaning. Rat pups, aged either 3, 5, 10, 12, 17 or 19 days at the beginning of the experiment, were placed in a chamber after daily injection with cocaine (7.5 or 15 mg/kg, i.p.) for either 2 or 4 consecutive days, and were tested for behavioral responsiveness to cocaine in the same chamber 24 h later (at either 7, 14 or 21 days of age). Such a short post-treatment interval was adopted, along with a consistent pairing of the testing context with the drug effect and a sensitive technique of behavioral measurement (video recording), in order to maximize the possibility of detecting any cocaine sensitization. Locomotion was sensitized at all ages, after both regimens in 14-day-old pups, but solely after 2 injections in 21- and 4 injections in 7-day-old pups. Sensitization was also expressed via behaviors specific to each age. Four cocaine injections augmented cocaine-induced uncoordinated movements of head, paws and body (horizontal activity) in 7-day-old pups, and mouth movements in 14-day-old pups. In 21-day-old pups, sensitization was dose- and regimen-dependently expressed via adult-like stereotyped head movements. In neonatal 7-day-old pups, cocaine sensitization was also visible as reductions in immobility (both injection regimens). Contrary to previous studies, these results indicate that, given the use of an appropriate methodology, short-term sensitization to the motoric effects of cocaine can be expressed by suckling rats prior to weaning, even after relatively short regimens of daily injections. © 1997 Elsevier Science B.V.

Keywords: Behavioral sensitization; Cocaine; Development, ontogeny; Neonatal rat; Preweanling rat

1. Introduction

Behavioral sensitization refers to the increase of the behavioral effects of a drug after its intermittent administration or to their progressive increment over successive injections. A relatively large number of regular injections, or high doses, of the drug can produce intense stereotyped oro-facial movements. It occurs, typically but not exclusively, with compounds having stimulant effects, such as post-synaptic dopamine agonists and indirect dopamine agonists such as cocaine, cocaine-like drugs and amphetamines (Post et al., 1992; Kalivas et al., 1993; Stewart and Badiani, 1993). Sensitization can be expressed several days, weeks or even months after cessation of the intermittent treatment, indicating long-term changes of the sites involved in its expression. Studies also suggest the existence of a short-term sensitization, which is expressed less than 48 h after treatment (Kalivas and Stewart, 1991; Zahniser and Peris, 1992; MacDougall et al., 1994; Heidebreder et al., 1996).

As regards ontogeny, the expression of long-term behavioral sensitization to psychomotor stimulants seems to be restricted to adult, juvenile, or at least weaned rats. In fact studies have suggested that sensitized responses will appear only if the intermittent administration of cocaine, amphetamine or methamphetamine commences at the end of the second or the third week of postnatal life (Sobrian et al., 1975; Fujiwara et al., 1987; Kolta et al., 1990; Scalzo and Holton, 1990; Barron et al., 1994; MacDougall et al., 1994; Tsuchida et al., 1994; Dow-Edwards and Hughes, 1995; Ujike et al., 1995). In these studies, rat pups were given 4 to 10 consecutive injections of a psychomotor stimulant either once or twice daily, sometimes beginning on the first day of postnatal life and the responsiveness to the pretreatment drug was acutely assessed after various periods of abstinence ranging from 2 days to several weeks.

* Corresponding author. Tel.: (32-4) 366-2018; Fax: (32-4) 366-2859; e-mail: ezio.tirelli@ulg.ac.be

Other results suggest that suckling rats are also unable to express either short- and long-term behavioral sensitization to cocaine, amphetamine or methamphetamine when tested before or during weaning, especially as neonatals (Sobrian et al., 1975; Kolta et al., 1990; Scalzo and Holton, 1990; Barr and Wang, 1993; Meyer and Yacht, 1993; Barron et al., 1994; MacDougall et al., 1994; Bowman and Kuhn, 1996). The drug was given 4 to 10 times, either once or twice daily, on postnatal days 1–20, and sensitization was tested on days 7, 10, 11, 14, 19, or 21–24, after an abstinence period of 1–15 days. Only two studies provided evidence of short-term sensitization in preweanlings. Pups aged 7 days showed increased effects of cocaine on ultrasonic vocalizations after daily cocaine injections on days 1–7 (Barr and Wang, 1993), and pups aged either 16 or 22 days expressed increased responsiveness (locomotion, head movements) to *d*-amphetamine last given 48 h earlier after 4 daily injections (MacDougall et al., 1994). Such discrepant data are rather in contrast with the well established capability of suckling rats to show behavioral sensitization to direct dopamine agonists, phencyclidine and caffeine (Guillet, 1990; Holloway, 1982; Scalzo and Holton, 1992; MacDougall et al., 1992, 1994; Kostrzewa, 1995).

In some of the above-mentioned studies that failed to reveal preweanling sensitization, cocaine or methamphetamine-pretreated pups exhibited slightly increased, but nonsignificant, levels of locomotion on the test day, suggesting that sensitization could well have occurred at early ages and might have been missed because of a lack of sensitivity in behavioral measurement. This may have been favored by the use of automatic activity meters and open-field matrix or rating scales, all of which are typical of behavioral pharmacology studies using adult animals. This feature is particularly important if one considers that the main components of quadruped locomotion do not emerge before the end of the second week of postnatal age, although fine motor coordination appears a week later (Westerga and Gramsbergen, 1990). It is therefore possible that the detection of sensitization was missed in the previous studies because pups expressed it through an age-specific form of locomotion (e.g., crawling and rolling movements) or via behaviors unrelated to locomotion (e.g., vertical movements directed toward the cage wall, head movements). Additionally, it is known that a consistent pairing of the injection environment with the drug effect throughout an intermittent treatment regimen can facilitate the establishment of sensitization; the effect size is often then greater than that obtained in animals injected in the colony room and tested in a different room (Post et al., 1992; Stewart and Badiani, 1993). In fact, the only study showing motoric sensitization prior to weaning was the only one to keep the environment constant over the experiment (MacDougall et al., 1994). Therefore, it may be that sensitization developed at levels too low to be reliably detected in the previous unsuccessful studies.

The purpose of the present study was to reexamine whether rat pups are able to express short-term sensitization to the behavioral effects of cocaine before weaning, including during the neonatal period. We tried to maximize the likelihood of detecting a sensitized effect by addressing the methodological limitations described above. Thus, pups' behavior was analyzed via videographic recording, which permits careful and repeated examination of behavior; the contextual cues of the experimental environment were kept constant across the intermittent treatment (either 2 or 4 daily injections of cocaine); and a short withdrawal interval of 24 h was adopted. Note that 1-day intervals are widely used in studies of behavioral sensitization with adult animals (e.g., Post et al., 1992; Terry, 1992; Kalivas and Duffy, 1993; Mattingly et al., 1994). The expression of sensitization was revealed when the behavioral response to cocaine shown by the cocaine-pretreated pups was greater than that of saline-pretreated animals, with a concomitant absence of an increase in pups pretreated with cocaine and tested under saline (i.e., following cocaine withdrawal).

2. Materials and methods

2.1. Subjects

We used a total of 336 pups taken from 48 litters and aged either 3, 5, 10, 12, 17 or 19 days. A vivarium of OFA-Sprague-Dawley rats (purchased from Iffa-Crédo, Onçins, France) was maintained under a light-dark cycle of 12/12 h (lights on 08.00 h), at a temperature of 22–26°C and humidity not controlled. Rats were kept in $40 \times 25 \times 20$ cm polypropylene tub cages with sawdust on the floors. One to three females were housed with one sire for breeding. Pregnant breeders were checked for births every day at approximately 10.00 and 17.00 h. A new litter was considered to be 0-day-old. When aged 2 days, pups and their dam were housed separately. In order to attenuate differential maternal effects on early ontogeny, and to facilitate treatment allocation within the litters, 2-day-old pups of the same weight were redistributed across litters such that each litter included 7–8 males and 2–3 females. Only the males were used.

2.2. Behavioral apparatus

Throughout the experiments, pups were placed individually in clear acrylic chambers, without bases (17×17 cm surface area). Four of these chambers were partitioned out of a single square box which was placed on a square platform of clear glass held horizontally by a robust frame. A removable wooden plate served as a lid. A portable S-VHS video camera (type Panasonic NS-MS1) was positioned directly underneath in order to view the whole surface covered by the chambers, an arrangement which

allowed filming of the four pups ventrally. A character generator (type Panasonic VW-CG2E) was connected to the control monitor and was used to indicate elapsed time ($100\ s^{-1}$). Illumination was provided by four neon tubes fixed singly on each leg of the frame. Each apparatus was individually located in a small, ventilated, white room (140×140 cm surface $\times$ 245 cm height) in which the ambient temperature was 22–26°C. Video tapes were marked and subsequently replayed for analysis on a high quality video cassette recorder that has slow-motion and frame-by-frame control options (type JVC HR-S5500E).

2.3. Experimental design and procedure

Cocaine hydrochloride (Belgopia, Louvain-La-Neuve, Belgium) was dissolved in physiological saline (0.9% NaCl) and injected i.p. The study included six subexperiments, each dealing with one of the three age groups (either 7, 14 or 21 on test day) and either 2 or 4 daily pretreatment injections. In each subexperiment, that included 7 possible drug conditions ($n = 8$), pups received a daily injection of either saline, 7.5 or 15 mg/kg cocaine and were tested for responsiveness to either saline, 7.5 or 15 mg/kg cocaine 24 h after the last of the pretreatment injections (see the respective figure legends for further details). Doses were chosen on the basis of pilot studies. The males of a litter were first divided in two groups of 3–4 pups and placed individually in the chamber, one per min, every day. On the fifth minute, pups were injected every minute and left in the chamber for 65–70 min. Filming started immediately befroe the placement of the first pup, on test day only. All behavioral testing was conducted between 9.00 and 18.00 h.

2.4. Behavioral quantification

The behavioral categories were identified in a first analysis of the video tapes. Pups were then scored for these behaviors using a multi-subject momentary sampling technique and a blind procedure. Each pup was scored over 14 samples of 1 min, beginning 4 min after cocaine injection and 8 min after being placed in the chamber, for a total post-injection period of 56 min. Thus, given that a video tape involved up to 4 pups that were observed singly in turn, 1 min per pup, this period involved 14 turns of 4 min each. Every 1-min sample was divided into 12 point samples for momentary sampling. On the moment of each point sample (first second of the 5-s interval), the observer recorded whether or not one of the targeted behaviors was occurring. A score of 1 (positive check) was assigned if the behavior was present and a score of 0 if it was absent (maximal score = 168). When only 3 pups were placed in the same arena, the fourth sampling min was maintained but without scoring. Only the most relevant behavioral categories for assessing sensitization are reported here, they were as follows:

(1) *Immobility*. In the 21- and 14-day-old pups, this behavior consists of standing, lying (with the legs retracted close to the body or stretched) and sleeping position; in the 7-day-old pups this consists of lying still on the flank or the back without any leg or head movement.

(2) *Mouthing*. Present in all age groups; attempts to gnaw the floor or the wall, licking the floor or the wall, nibbling the paws maintained on the floor.

(3) *Head scanning*. Stereotyped movements of the head, either up and down directed toward the wall (head bobbing), or side-to-side motions (head weaving), with the nose frequently contacting the floor; visible only in 21-day-old pups.

(4) *Rearing*. Body vertically or nearly vertically maintained with the forelimbs in the air or in contact with the wall, either with or without obvious sniffing; occurs in such a coordinated form in 21- and 14-day-old pups.

(5) *Vertical activity*. Present only in 7-day-old pups; included attempts to climb the wall with paw movements (scrabbling), leaning on the wall with at least one paw was also included; the body was usually arched and was maintained vertically.

(6) *Horizontal activity*. Present only in 7-day-old pups; this included most of the movements that a neonatal rat typically shows when placed on a smooth floor: uncoordinated crawling and scrabbling movements; rolling with the body on the floor often as a consequence of these movements; clonic body twitches, lateral head movements and head-raising.

Another behavior, locomotion, was scored separately. It was defined as the displacement of the body from a given location of the arena to another. With pieces of black tape fixed to the monitor, the surfaces of the chambers were divided into 4 or 9 equal squares in order to measure locomotor activity of 21-day-old or 14- and 7-day-old pups, respectively. The number of line crossings (four paws traversing the tape strip) was recorded continuously within each 1-min sample.

2.5. Data analysis

Since this study aimed at detecting the expression of sensitization at early ages, no statistical comparison between ages (7, 14 or 21 days) was included in the design. Direct comparisons of behaviors that belong to age-specific ethograms would indeed be misleading, even in the case of functionally equivalent or almost-equivalent behaviors such as locomotion or oral movements. Rather, the a priori Dunn's test, which is based on the additive Bonferroni unequality, was carried out to test the reliability of differences between the cocaine-plus-cocaine and the saline-plus-cocaine groups, with the saline-plus-saline animals as the unique control group (Marascuilo and Serlin, 1988). Comparisons between doses within age groups and across regimens within a given dose were also planned, thus yielding a total of 12 nonorthogonal pairwise comparisons.

To adjust for the absence of a consistent homogeneity of variances, the Welch-Aspin correction, which implies an adjustment of the degrees of freedom, was also computed. For the sake of clarity, only the final statistical significance, which was accepted at a probability level of 0.05, is given in the text.

3. Results

3.1. Effects of 2 prior daily injections of cocaine in 21-day-old pups (Fig. 1)

Cocaine pretreatment potentiated locomotion in cocaine-injected pups on the test day (7.5 mg/kg at $P < 0.05$ and 15 mg/kg at $P < 0.01$), sensitization being stronger in pups treated with 15 mg/kg cocaine, but not significantly so. At 7.5 mg/kg, acute cocaine significantly induced head scanning in cocaine-pretreated pups ($P < 0.05$). A greatest, 2- to 2.5-fold increment, in this stereotyped behavior was shown by the pups receiving 15 mg/kg cocaine ($P < 0.01$). Rearing did not undergo any substantial change after intermittent cocaine. Immobility was not altered by cocaine pretreatment either, probably because of the almost complete suppression of this behavior (further suppression, which was not possible, would have reflected sensitization).

3.2. Effects of 4 prior daily injections of cocaine in 21-day-old pups (Fig. 2)

Cocaine-induced locomotor stimulation was not changed substantially by prior intermittent cocaine, at either dose. On cocaine withdrawal, pups pretreated with 15 mg/kg cocaine showed significantly increased levels of locomotion ($P < 0.05$). Head scanning was elicited after 7.5 mg/kg and augmented following 15 mg/kg acute cocaine, in cocaine-pretreated pups (both doses at $P < 0.01$). This stereotyped behavior underwent a 12- to 13-fold increase in 15 mg/kg cocaine-pretreated pups, which showed significantly higher levels than those similarly treated with 7.5 mg/kg cocaine ($P < 0.01$). Although 15 mg/kg cocaine decreased rearing, 7.5 mg/kg cocaine did not affect that behavior in cocaine-pretreated pups ($P < 0.05$). Acute cocaine suppressed immobility in all the saline-pretreated pups ($P < 0.01$), such that a further in-

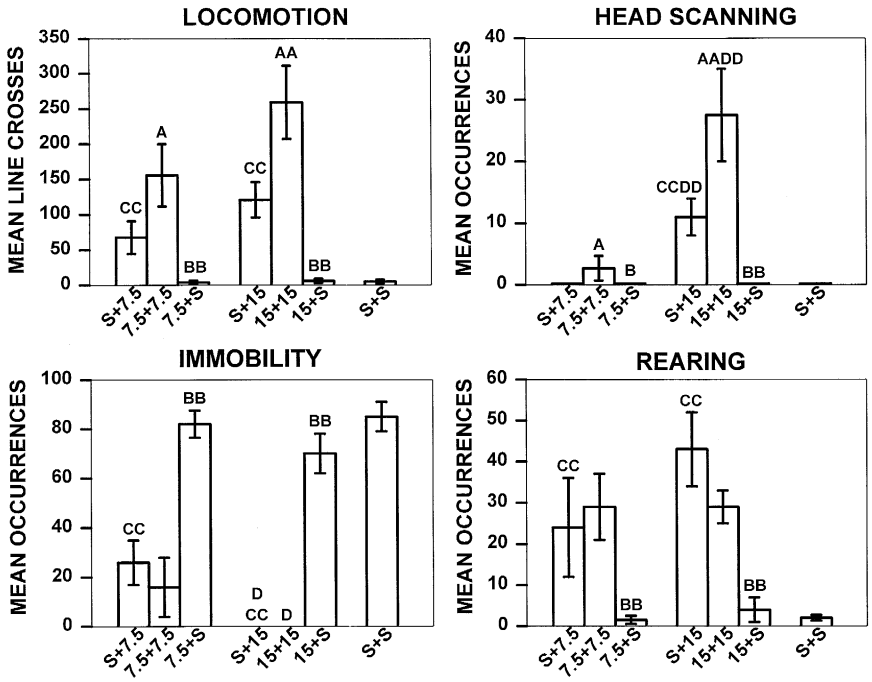

Fig. 1. Effects of acute cocaine (either saline, 7.5 or 15 mg/kg) on locomotion, head scanning, immobility and rearing in 21-day-old rat pups having received, 24 h earlier, the last of 2 daily i.p. injections of either 7.5 or 15 mg/kg cocaine. S + 7.5: group pretreated with saline and challenged on test day with 7.5 mg/kg cocaine; 7.5 + 7.5: group pretreated with 7.5 mg/kg cocaine and challenged on test day with 7.5 mg/kg cocaine; 7.5 + S: group pretreated with 7.5 mg/kg cocaine and challenged on test day with saline; S + 15: group pretreated with saline and challenged on test day with 15 mg/kg cocaine; 15 + 15: group pretreated with 15 mg/kg cocaine and challenged on test day with 15 mg/kg cocaine; 15 + S: group pretreated with 15 mg/kg cocaine and challenged on test day with saline; S + S: group receiving saline as pretreatment and saline on test day. A or AA: different from the respective saline-plus-cocaine group at $P < 0.05$ or $P < 0.01$, respectively; B or BB: different from the respective cocaine-plus-cocaine group at $P < 0.05$ or $P < 0.01$, respectively; C or CC: different from the saline-plus-saline group at $P < 0.05$ or $P < 0.01$, respectively; D or DD: different from the smaller cocaine dose at $P < 0.05$ or $P < 0.01$, respectively. For clarity, the differences across regimen within doses are not marked but described in the text when necessary. Note that locomotion was scored as matrix crossings and the other behaviors via a momentary time-sampling technique with a theoretical maximal score of 168 occurrences (see Section 2). Values are means and vertical brackets represent standard errors of the mean ($n = 8$).

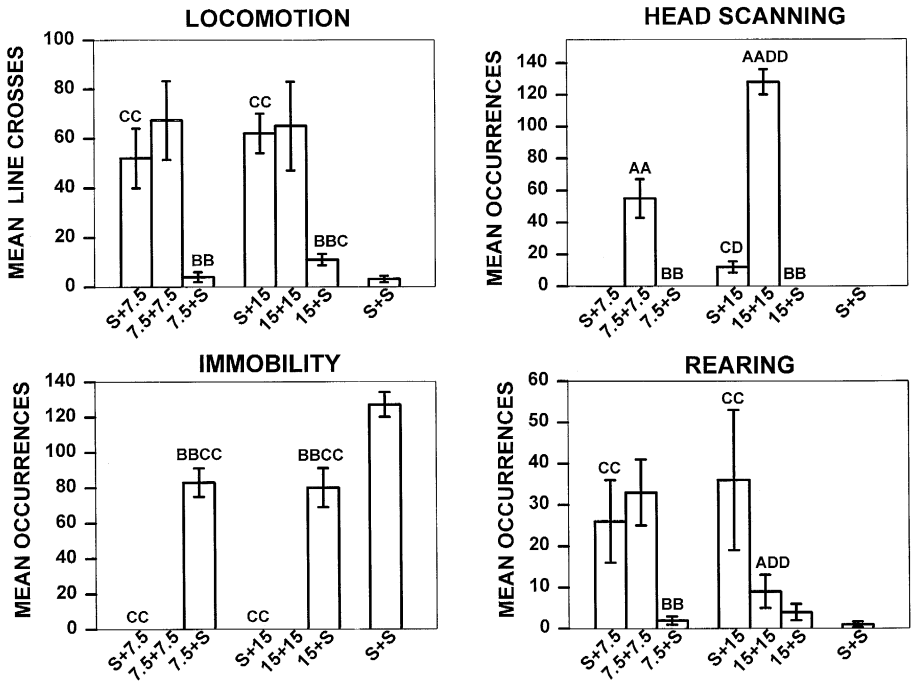

Fig. 2. Effects of acute cocaine (either saline, 7.5 or 15 mg/kg) on locomotion, head scanning, immobility and rearing in 21-day-old rat pups having received, 24 h earlier, the last of 4 daily i.p. injections of either saline, 7.5 or 15 mg/kg cocaine. S + 7.5: group pretreated with saline and challenged on test day with 7.5 mg/kg cocaine; 7.5 + 7.5: group pretreated with 7.5 mg/kg cocaine and challenged on test day with 7.5 mg/kg cocaine; 7.5 + S: group pretreated with 7.5 mg/kg cocaine and challenged on test day with saline; S + 15: group pretreated with saline and challenged on test day with 15 mg/kg cocaine; 15 + 15: group pretreated with 15 mg/kg cocaine and challenged on test day with 15 mg/kg cocaine; 15 + S: group pretreated with 15 mg/kg cocaine and challenged on test day with saline; S + S: group receiving saline as pretreatment and saline on test day. Other details as described for Fig. 1.

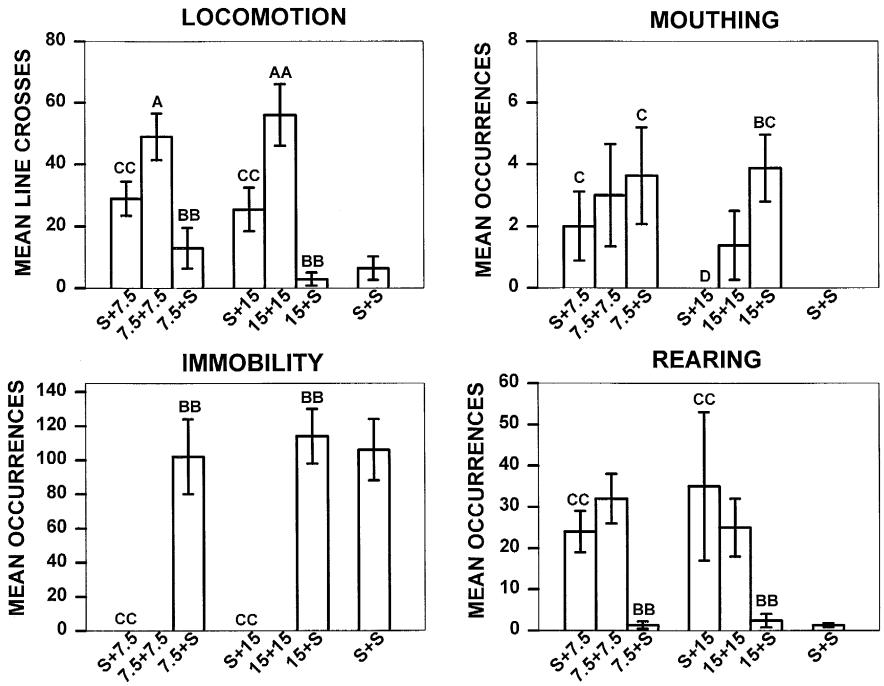

Fig. 3. Effects of acute cocaine (either saline, 7.5 or 15 mg/kg) on locomotion, mouthing, immobility and rearing in 14-day-old rat pups having received i.p., 24 h earlier, the last of 2 daily i.p. injections of either saline, 7.5 or 15 mg/kg cocaine. S + 7.5: group pretreated with saline and challenged on test day with 7.5 mg/kg cocaine; 7.5 + 7.5: group pretreated with 7.5 mg/kg cocaine and challenged on test day with 7.5 mg/kg cocaine; 7.5 + S: group pretreated with 7.5 mg/kg cocaine and challenged on test day with saline; S + 15: group pretreated with saline and challenged on test day with 15 mg/kg cocaine; 15 + 15: group pretreated with 15 mg/kg cocaine and challenged on test day with 15 mg/kg cocaine; 15 + S: group pretreated with 15 mg/kg cocaine and challenged on test day with saline; S + S: group receiving saline as pretreatment and saline on test day. Other details as described for Fig. 1.

crease in immobility resulting from cocaine pretreatment could not be expressed. Interestingly, immobility was substantially reduced in pups pretreated with cocaine and tested under saline ($P < 0.01$), with no significant difference between these two effects.

Some of the sensitized effects were regimen-dependent. At comparable control levels, head scanning by pups receiving 4 injections of 15 mg/kg cocaine was 5-fold greater than was induced by the 2-injection regimen; similar comparisons can be made for the lower dose of cocaine (all contrasts across regimens significant at $P < 0.01$). Also, 2-injection-treated pups showed less immobility than those injected and exposed to the testing environment 4 times prior to testing ($P < 0.01$).

3.3. Effects of 2 prior daily injections of cocaine in 14-day-old pups (Fig. 3)

For both acute cocaine doses, cocaine-pretreated pups exhibited significantly higher levels of locomotion as compared to the saline-pretreated pups (7.5 mg/kg at $P < 0.05$ and 15 mg/kg at $P < 0.01$). This effect was slightly stronger in pups receiving 15 mg/kg cocaine (2-fold increase). Few, nonsignificant, episodes of mouthing were induced by cocaine in cocaine-pretreated pups (both doses). However, these pups mouthed significantly more under saline than under the test doses of cocaine ($P < 0.01$). Rearing was not substantially changed by the cocaine pretreatments and only a larger variability was found for 15 mg/kg cocaine. Immobility was completely suppressed by acute cocaine at both doses in saline-pretreated pups ($P < 0.01$), as well as in those pretreated with cocaine.

3.4. Effects of 4 prior daily injections of cocaine in 14-day-old pups (Fig. 4)

Under acute cocaine, cocaine-pretreated pups showed levels of locomotion significantly higher than those in saline-pretreated pups, at both doses (7.5 mg/kg at $P < 0.05$ and 15 mg/kg at $P < 0.01$). The effects of the higher cocaine dose were the strongest but not significantly so. At both doses, cocaine pretreatment potentiated cocaine-induced mouthing (at $P < 0.01$ for the lower, and at $P < 0.05$ for the higher dose), the increment due to 7.5 mg/kg cocaine being greater. Note that this occurred solely following the longest injection regimen. Whereas the pretreatment with 7.5 mg/kg cocaine did not change cocaine-induced rearing activity, pretreatment with 15 mg/kg cocaine significantly decreased this effect (at $P < 0.05$). Pups pretreated with cocaine either acutely or intermittently showed no sign of immobility, at either dose (all at $P < 0.01$). This feature clearly impeded the potential detection of sensitization. Cocaine withdrawal significantly decreased immobility at both cocaine doses ($P < 0.01$) and rearing at 15 mg/kg cocaine ($P < 0.05$).

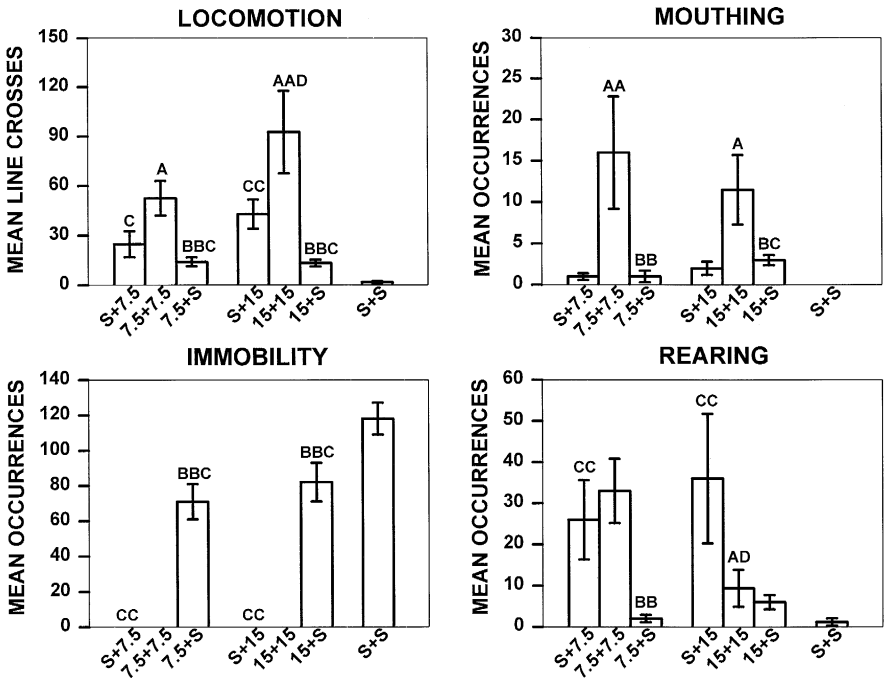

Fig. 4. Effects of acute cocaine (either saline, 7.5 or 15 mg/kg) on locomotion, mouthing, immobility and rearing in 14-day-old rat pups having received, 24 h earlier, the last of 4 daily i.p. injections of either saline, 7.5 or 15 mg/kg cocaine. S + 7.5: group pretreated with saline and challenged on test day with 7.5 mg/kg cocaine; 7.5 + 7.5: group pretreated with 7.5 mg/kg cocaine and challenged on test day with 7.5 mg/kg cocaine; 7.5 + S: group pretreated with 7.5 mg/kg cocaine and challenged on test day with saline; S + 15: group pretreated with saline and challenged on test day with 15 mg/kg cocaine; 15 + 15: group pretreated with 15 mg/kg cocaine and challenged on test day with 15 mg/kg cocaine; 15 + S: group pretreated with 15 mg/kg cocaine and challenged on test day with saline; S + S: group receiving saline as pretreatment and saline on test day. Other details as described for Fig. 1.

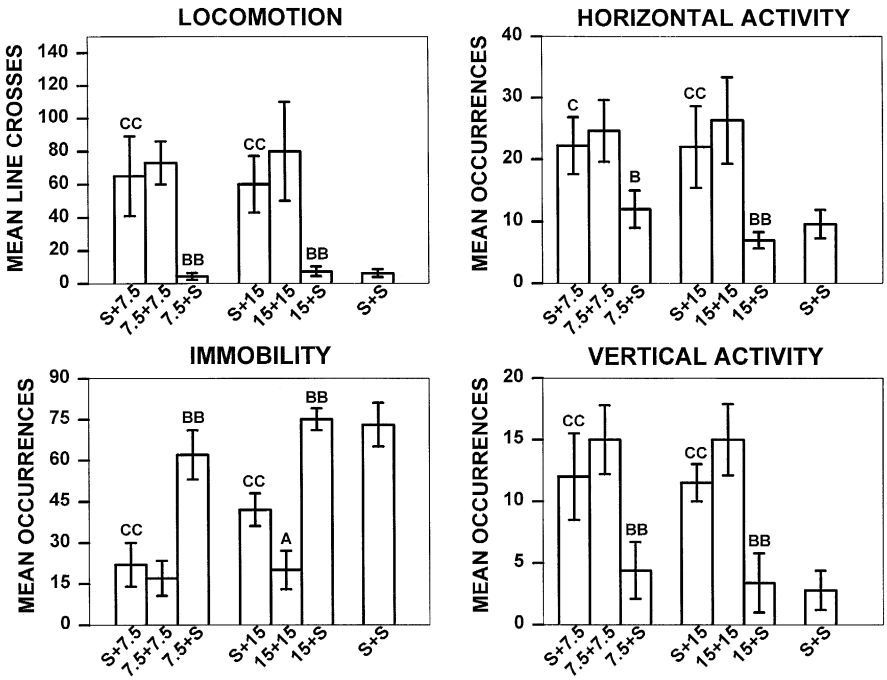

Fig. 5. Effects of acute cocaine (either saline, 7.5 or 15 mg/kg) on locomotion, horizontal activity, immobility and vertical activity (see Section 2 for a precise description of these behavioral categories) in 7-day-old rat pups having received, 24 h earlier, the last of 2 daily i.p. injections of either saline, 7.5 or 15 mg/kg cocaine. S + 7.5: group pretreated with saline and challenged on test day with 7.5 mg/kg cocaine; 7.5 + 7.5: group pretreated with 7.5 mg/kg cocaine and challenged on test day with 7.5 mg/kg cocaine; 7.5 + S: group pretreated with 7.5 mg/kg cocaine and challenged on test day with saline; S + 15: group pretreated with saline and challenged on test day with 15 mg/kg cocaine; 15 + 15: group pretreated with 15 mg/kg cocaine and challenged on test day with 15 mg/kg cocaine; 15 + S: group pretreated with 15 mg/kg cocaine and challenged on test day with saline; S + S: group receiving saline as pretreatment and saline on test day. Other details as described for Fig. 1.

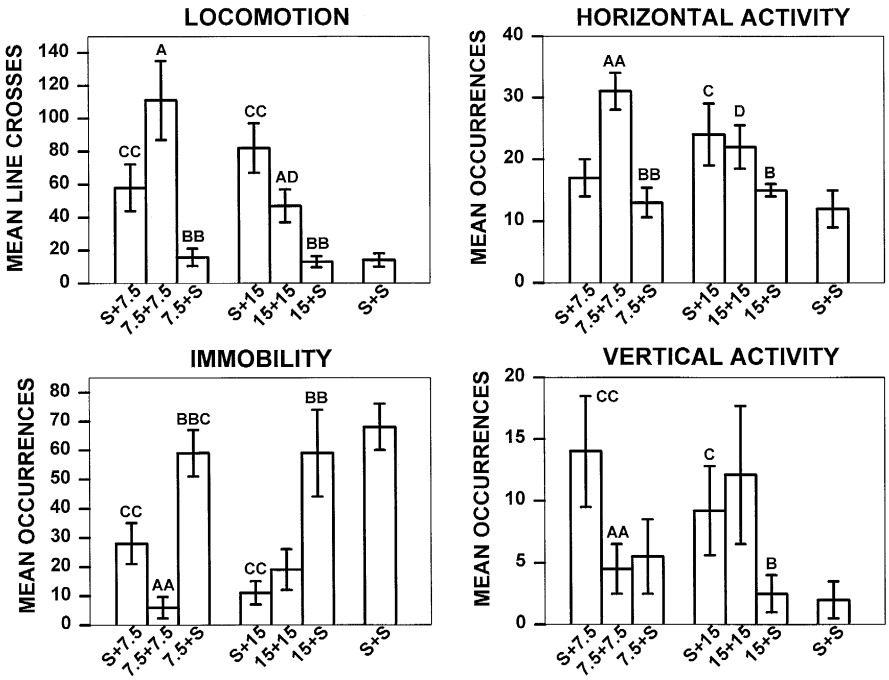

Fig. 6. Effects of acute cocaine (either saline, 7.5 or 15 mg/kg) on locomotion, horizontal activity, immobility and vertical activity (see Section 2 for a precise description of these behavioral categories) in 7-day-old rat pups having received, 24 h earlier, the last of 2 daily i.p. injections of either saline, 7.5 or 15 mg/kg cocaine. S + 7.5: group pretreated with saline and challenged on test day with 7.5 mg/kg cocaine; 7.5 + 7.5: group pretreated with 7.5 mg/kg cocaine and challenged on test day with 7.5 mg/kg cocaine; 7.5 + S: group pretreated with 7.5 mg/kg cocaine and challenged on test day with saline; S + 15: group pretreated with saline and challenged on test day with 15 mg/kg cocaine; 15 + 15: group pretreated with 15 mg/kg cocaine and challenged on test day with 15 mg/kg cocaine; 15 + S: group pretreated with 15 mg/kg cocaine and challenged on test day with saline; S + S: group receiving saline as pretreatment and saline on test day. Other details as described for Fig. 1.

3.5. Effects of 2 prior daily injections of cocaine in 7-day-old pups (Fig. 5)

Cocaine-induced locomotor stimulation was not changed by cocaine pretreatment at any test treatment. Similarly, neither horizontal activity nor vertical activity was affected by cocaine pretreatments. The decrease in immobility induced by 15 mg/kg cocaine was significantly potentiated in pups pretreated with the same dose of cocaine, indicating sensitization ($P < 0.05$). At 7.5 mg/kg, cocaine pretreatment did not induce any further decrease in immobility.

3.6. Effects of 4 prior daily injections of cocaine in 7-day-old pups (Fig. 6)

Cocaine-induced locomotor activity was not changed significantly by intermittent cocaine at the dose of 15 mg/kg, whereas it underwent a significant, almost 2-fold, increment in pups pretreated with 7.5 mg/kg cocaine ($P < 0.05$). A similar pattern of effects was obtained for horizontal activity, for which pretreatment of 7.5 mg/kg cocaine significantly increased acute cocaine effects ($P < 0.01$). This pattern of effects also occurred for vertical activity but only at the 15 mg/kg dose. Under acute cocaine, 7.5 mg/kg cocaine showed significantly less vertical activity than in those pretreated with saline ($P < 0.05$). Cocaine pretreatment accentuated cocaine-induced immobility decreases, significantly at 7.5 mg/kg but not so at 15 mg/kg cocaine ($P < 0.01$). Note that the difference consisted of an almost 5-fold decrement (values from 28.6 to 6.2), suggesting a robust effect.

3.7. Summary of the results (Table 1)

Behavioral sensitization occurred in all age groups. In 21-day-old pups, head scanning was sensitized at both regimens (both doses), and locomotion after the 2-injection regimen (both doses) only. Pups aged 14 days expressed sensitization via locomotion after both regimens (both doses) and via mouthing after the 4-injection regimen (both doses). In 7-day-old pups, sensitization was more visible after the 4-injection regimen for which three behavioral instances were increased (locomotion, immobility and horizontal activity; at the lowest dose), instead of one after the 2-injection regimen (immobility, at the highest dose). As a corollary to sensitization-related increments, some behaviors such as rearing and vertical activity, were either reduced probably via behavioral competition or remained unaffected in all age groups.

4. Discussion

The major finding of the current study is that sensitization to the behavioral effects of cocaine can be expressed by preweanling rats, including neonatal 7-day-old pups, having received as few as 2 daily pre-injections of cocaine the day before. Whereas locomotion was the only behavior that was sensitized in all age groups, sensitization also occurred for several behavioral categories which can be considered age-specific (horizontal activity, which included several uncoordinated movements, in 7-day-old pups, and adult-like head scanning in 21-day-old pups). It seems that consistent regimen- and dose-related sensitization, as well as acute cocaine effects (saline-pretreated pups), became increasingly visible as the pups matured. Plausibly, this could result from the relative incompleteness of motoric differentiation in the neonatal pups, impeding the use of variable and fine movements to behaviorally translate the effects of gradual neuropharmacological mobilizations. Moreover, these limitations might have been somewhat potentiated by the higher neonatal susceptibility to cocaine, which may include excessive anesthetic and

Table 1
Summary of the behavioral effects of an acute injection of cocaine (either 7.5 or 15 mg/kg) in 21-, 14- and 7-day-old pups having received either 4 or 2 daily injections of cocaine (either 7.5 or 15 mg/kg) 24 h earlier

Age on test day	21-day-old pups		14-day-old pups		7-day-old pups	
Daily injections	4	2	4	2	4	2
Locomotion	No [a]	YES [b]	YES	YES	YES	No
Immobility (decrease)	No	No	No	No	YES	YES
Head scanning	YES	YES	Rare [c]	Rare	Absent [d]	Absent
Rearing	No	No	No	No	Absent	Absent
Mouthing	Rare	Rare	YES	No	Rare	Rare
Horizontal activity	Absent	Absent	Absent	Absent	YES	No
Vertical activity	Absent	Absent	Absent	Absent	No	No

[a] Absence of a sensitizing effect.
[b] Occurrence of sensitization for at least one of the two cocaine doses (significant difference between the saline-plus-cocaine and cocaine-plus-cocaine groups for at least one of the cocaine doses with no contribution of a cocaine withdrawal effect).
[c] Behavior rarely observed (less than three occurrences).
[d] Behavior never observed or known to be absent in the individual ethogram of pups from that particular age group (see Section 2 for a precise description of the behavioral categories).

depressive pre-convulsant effects (note that no pup in this study showed signs of convulsions).

Control cocaine-pretreated pups sometimes were slightly but significantly stimulated under saline (locomotion and immobility in 21- and 14-day-old pups, mouthing in 14-day-old pups, cf., Figs. 2–4), especially for the longer regimen. It is likely that these differences reflected a conditioned drug effect rather than an abstinence effect, since cocaine abstinence has been shown to induce behavioral depression (Costall et al., 1990; Bozarth, 1989; Post et al., 1992). The magnitude of these effects on locomotion and mouthing was not sufficient to account for the differences reflecting sensitized effects on these behaviors. In contrast, for immobility, the decreases on withdrawal were relatively substantial (at both doses), without any sensitization being visible on it. Although sensitization on immobility might have been obscured by a floor effect, it is possible that the expression of the withdrawal effect was independent from sensitization. Whichever effect our cocaine withdrawal really produced, it remains the case that preweanlings are able to express neurobehavioral phenomena that are related to cocaine chronic administration, additionally to sensitization.

Our results are not in accordance with the previous ontogenetic studies, where preweanlings did not express significant sensitization to the motoric effects of cocaine given over 4–10 days (Barr and Wang, 1993; Meyer and Yacht, 1993; Scalzo and Holton, 1990; Barron et al., 1994; Bowman and Kuhn, 1996). As suggested in Section 1, a critical factor facilitating the detection of cocaine-induced behavioral sensitization in preweanling pups is likely to be the sensitivity and the appropriateness of the measurement technique. The procedure for measuring behavior here was different in various respects from procedures adopted previously. In some studies, for example, the duration of measurement was only of 5–6 min (Barr and Wang, 1993; Meyer and Yacht, 1993). Considering that cocaine's effects do not necessarily emerge immediately after injection, even after repeated injections, too short an observation period might have led to missing the full-blown behavioral response. Perhaps more importantly, in the previous studies behavior was scored visually using a rating scale or quadrant movements (locomotion), combined with either a continuous or a time-sampled observation session of observation. One study utilized voltage sensors, presumably to record total movements. In the studies using counts of matrix crosses to measure locomotor activity (Barr and Wang, 1993), as we did here, pups were placed in a small testing chamber with a smooth floor divided into 6–9 squares (4–5 cm side). It is clear that the criterion used to register the square entries, which was not always specified in the previous studies, may have been critical for the locomotion score. This is particularly important given that neonatal rats, whose quadrupedal locomotion is not yet developed (Westerga and Gramsbergen, 1990), exhibit much pivoting and crawling movements which do not necessarily contribute to a complete translocation of the body (from one point of the arena to another). For example, recording one locomotor count whenever the pup fully changes square, with the whole body, or only when its head and the forepaws intersect the crossing line, as we did here, will likely yield different locomotor scores. Additionally, the usual criticisms addressed to rating scales (Rebec and Bashore, 1984; Cooper and Dourish, 1990) obviously hold for suckling rats too; such assessments can only reflect a general intensity effect of the drug, collapsing different behavioral changes that might have revealed sensitization if scored separately (e.g., Bowman and Kuhn, 1996).

In the current study, we scored video records, an approach that allowed us to identify and analyze the behavioral categories more reliably than with direct visual scoring or even actographic recordings. A time-sampling technique was then applied to quantify behaviors from these categories (defined a posteriori). Moreover, and of particular importance, pups were filmed from a ventral view point, the camera being positioned directly under the transparent floor of the test chamber. With such a procedure, limb- and orofacial-related behaviors could be analyzed adequately, something that is not always possible when the animals are scored using the conventional dorsal and lateral views. Therefore, it is likely that sensitization on mouthing in 14-day-old pups would not have been detected using such angles of observation. Also, the unambiguous identification of line crossings, which are perfectly visible from the ventral perspective, clearly facilitated the detection of drug-induced locomotor changes.

Consistent pairings of the drug effect with the context in which the animals are placed post-injection can facilitate behavioral sensitization, most likely by means of conditioning processes (Hinson and Poulos, 1981; Post et al., 1992; Stewart and Badiani, 1993), and such a contextual facilitation may have occurred in our study. This is indirectly supported by the stimulation observed in some of our cocaine-withdrawn pups, admitting that this effect actually reflected a conditioning response. That suckling rats and mice do, in fact, show conditioned place preference for cocaine, where the environmental cues paired with drug action are actively sought by the pups, suggests that this might well be the case (Barr and Wang, 1992; Laviola et al., 1992). Even more significantly, the only demonstration of sensitization to the motoric effects of cocaine in preweanlings has been obtained by pairing the drug with the context over the intermittent treatment (MacDougall et al., 1994). No pups pretreated outside the test room were included in the design, so it remains unclear whether the context-drug pairing was indispensable for sensitization to be expressed by the preweanlings. However, it might be noted that a number of reports indicate that where the absence of such pairings attenuates the development of sensitization it does not necessarily impede it. Indeed, robust behavioral sensitization has been

readily obtained in adult animals acutely or intermittently pretreated with cocaine in their home cage before being challenged with cocaine in a separate test room, one, two or several days later (Gale, 1984; Lin-Chu et al., 1985; De Montis et al., 1992; Post et al., 1992; Heidebreder et al., 1993; Hooks et al., 1993; Kalivas and Duffy, 1993; Mattingly et al., 1994).

In the current study, pups were tested 24 h after cocaine pretreatment, which may have favored the expression of sensitization. Comparable and even shorter post-treatment intervals were used in some of the previous unsuccessful studies, testing cocaine and *d*-amphetamine in suckling rats (e.g., Sobrian et al., 1975; Barr and Wang, 1993; Meyer and Yacht, 1993; Bowman and Kuhn, 1996). Sensitization did not occur either when pups were tested after abstinence periods lasting more than 9–10 days (Kolta et al., 1990; Scalzo and Holton, 1990; Barron et al., 1994; Ujike et al., 1995). The length of the post-treatment intervals cannot therefore be taken as a convincing source of discrepancy between these studies and ours. However, that the withdrawal interval plays a role in the duration of sensitized effects in preweanling rats is suggested by the recent work by MacDougall et al. (1994), where preweanling pups showed sensitization 2 but not 8 days after intermittent *d*-amphetamine. Differential pharmacological blockade of either the establishment of sensitization or its persistence suggests that dissimilar neural mechanisms underlie short-term and long-term sensitization, the former (lasting less than 48 h) being reversible whereas the latter is more likely to be irreversible (Kalivas and Stewart, 1991; Zahniser and Peris, 1992; Heidebreder et al., 1996). In this context, it is tempting to speculate that the failure to obtain long-term sensitization in infant rats is indicative of an immaturity of the corresponding neural, especially dopaminergic, mechanisms. Consistently, dopamine striatal release, uptake mechanisms and receptors are not fully developed before weaning in rats; and some dissimilar or even paradoxical neurophysiological processes have been identified in the neonatal rat's mesocorticolimbic and nigrostriatal areas, which are thought to subserve sensitization (e.g., Broaddus and Bennett, 1990; Le et al., 1992; Rao et al., 1991; Zahniser and Peris, 1992; Kalivas et al., 1993; Tepper et al., 1994; Tsuchida et al., 1994, 1996). The present results imply that these mechanisms are sufficiently functional for short-term sensitization to be expressed during the neonatal period. At first sight, such a view is not in accordance with some of the available results indicating an increased responsiveness to cocaine or methamphetamine in adult or juvenile rats neonatally treated with these drugs (e.g., Hayashi et al., 1987; Dow-Edwards and Hughes, 1995). It must be kept in mind, however, that in these studies pups received a relatively intensive treatment during a period of rapid brain growth, such that it is unclear whether the observed effects were due to interferences with early ontogeny or long-lasting behavioral sensitization. Anyhow, this overall pattern of results is rather inconsistent and warrants a systematic reexamination of the post-treatment intervals, especially for clarifying the limits and the relationships between short-term and long-term sensitization as well as between the latter phenomenon and long-term developmental drug effects.

Neonatal rats are poikilothermic organisms and outside the nest (33°C) their core temperature and baseline behavioral activity strongly decrease. Thus, the situation in which our pups were tested (22–26°C) can be considered an 'extreme cold exposure' (Blumberg and Stolba, 1996). Given that this was also the case in the above-mentioned unsuccessful ontogenetic studies using cocaine, the occurrence of sensitization in the present study cannot be reasonably ascribed to differences in ambient temperature. Consistently, behavioral sensitization to the direct dopamine agonist *R*(−)-propylnorapomorphine has been obtained in 16- to 17-day-old rats tested either in an incubator at 31°C or in the laboratory room (MacDougall et al., 1992, 1994). Therefore, if a warm context potentiates the acute stimulant effects of dopamine agonists in neonatal rats (e.g., Camp and Rudy, 1987), it does not influence the likelihood for these to show behavioral sensitization to similar compounds.

It seems that pharmacokinetic factors contribute to the establishment of behavioral sensitization to cocaine injected i.p. or s.c., but not i.v., via mechanisms outside of blood and brain compartments (Pan et al., 1991; Pettit and Pettit, 1994). Specifically, high cocaine concentrations in both plasma and relevant brain areas can coexist in rodents examined 24 h after 1–10 daily cocaine injections or even 7 days after a single administration of cocaine, the persistence of behavioral sensitization being closely paralleled by these pharmacokinetic parameters (Pan et al., 1991; Cass and Zahniser, 1993; Pettit and Pettit, 1994). Obviously, these effects might hold for suckling rats as well, the more so as it is likely that the mechanisms of drug penetration and disposition are probably not as efficient in pups as in adults. To our knowledge, there is no available data on the pharmacokinetics of cocaine intermittently given to suckling rodents. Anyhow, the issue of whether or not behavioral sensitization in our pups is dependent upon pharmacokinetic factors is not, in fact, of great importance to our current purpose, since these factors contribute to an explanation of sensitization regardless of the age. It remains the case that, behaviorally, our pups are capable of expressing increased levels of stimulation whatever the somatic mechanisms could be. Therefore, the identification of the cerebral sites subserving behavioral sensitization in the suckling rat should only be studied using i.v. administration, since blood and brain cocaine concentrations do not increase after a cocaine challenge given via that route of administration (Pan et al., 1991; Pettit and Pettit, 1994). This is likely to be difficult to achieve in small-sized and quickly maturing animals.

In conclusion, this study is, to our knowledge, the first

report of short-term sensitization to the behavioral, motoric, effects of cocaine after relatively short intermittent treatments in preweanling and neonatal rats. Such an early maturation may not only provide an opportunity to dissect the mechanisms of sensitization, but may also assist our understanding of the ontogenetic determinants of cocaine abuse, for example by means of experiments that probe changes in drug abuse vulnerability precociously in pups born to experimentally addicted mothers.

Acknowledgements

E.T. is a research associate under contract with the Fonds National de la Recherche Scientique (FNRS, Belgium). This study was supported by the F.N.R.S. (grants F.R.S.M. No. 9.4568.93 and F.R.F.C. No. 2.4506.94). We thank Christian Heidebreder (Swiss Federal Institute of Technology at Zurich, Switzerland) and Phil Terry (University of Birmingham, UK) for the critical reading of the manuscript.

References

Barr, G.A., Wang, S., 1992. Peripheral and central administration of cocaine produce odor preferences in the infant rat. Brain Res. 599, 181.

Barr, G.A., Wang, S., 1993. Behavioral effects of chronic cocaine treatment in the week-old rat. Eur. J. Pharmacol. 233, 143.

Barron, S., Kaiser, D.H., Hansen, L.S., 1994. Neonatal cocaine exposure, activity, and responsivity to cocaine in a rodent model. Neurotoxicol. Teratol. 16, 401.

Blumberg, M.S., Stolba, M.A., 1996. Thermogenesis, myoclonic twitching, and ultrasonic vocalization in neonatal rats during moderate and extreme cold exposure. Behav. Neurosci. 110, 305.

Bowman, B.P., Kuhn, C.M., 1996. Age-related differences in the chronic and acute response to cocaine in the rat. Dev. Psychobiol. 29, 597.

Bozarth, M.A., 1989. New perspectives on cocaine addiction: recent findings from animal research. Can. J. Physiol. Pharmacol. 67, 1158.

Broaddus, W.C., Bennett, J.P., 1990. Postnatal development of striatal dopamine function. I. An examination of D_1 and D_2 receptors, adenylate cyclase regulation and presynaptic dopamine markers. Dev. Brain Res. 52, 265.

Camp, L.L., Rudy, J.W., 1987. Behavioral activation in infant rats: pharmacological evidence for dopaminergic mediation. Psychobiology 15, 317.

Cass, W.A., Zahniser, N.R., 1993. Cocaine levels in striatum and nucleus accumbens: augmentation following challenge injection in rats withdrawn from repeated cocaine administration. Neurosci. Lett. 152, 177.

Cooper, S.J., Dourish, C.T. (Eds.), 1990. Neurobiology of Stereotyped Behaviour. Oxford University Press, Oxford.

Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tyers, M.B., 1990. Ondansetron inhibits a behavioral consequence of withdrawing from drugs of abuse. Pharmacol. Biochem. Behav. 36, 339.

De Montis, M.G., Devoto, P., Meloni, D., Gambarana, C., Giorgi, G., Tagliamonte, A., 1992. NMDA receptor inhibition prevents tolerance to cocaine. Pharmacol. Biochem. Behav. 42, 179.

Dow-Edwards, D.L., Hughes, H.E., 1995. Adult reactivity in rats exposed to cocaine during two early postnatal periods. Neurotoxicol. Teratol. 17, 553.

Fujiwara, Y., Kazawaya, Y., Nakashima, M., Sato, M., Otuki, S., 1987. Behavioral sensitization to methamphetamine in the rat: an ontogenic study. Psychopharmacology 91, 316.

Gale, K., 1984. Catecholamine-independent behavioral and neurochemical effects of cocaine in rats. NIDA Res. Monogr. Ser. 54, 323.

Guillet, R., 1990. Neonatal caffeine exposure alters adenosine receptor control of locomotor activity in the developing rat. Dev. Pharmacol. Ther. 15, 94.

Hayashi, T., Kunihara, M., Tadokoro, S., 1987. Behavioral and neurochemical changes produced by postnatal pretreatments with methamphetamine in rats. Jpn. J. Pharmacol. 43, 17.

Heidebreder, C.A., Goldberg, S.R., Shippenberg, T.S., 1993. The kappa-opioid receptor agonist U-69593 attenuates cocaine-induced behavioral sensitization in the rat. Eur. J. Pharmacol. 616, 335.

Heidebreder, C.A., Thompson, A.C., Shippenberg, T.S., 1996. Role of extracellular dopamine in the initiation and long-term expression of behavioral sensitization to cocaine. J. Pharmacol. Exp. Ther. 278, 490.

Hinson, R.E., Poulos, C.X., 1981. Sensitization to the behavioral effects of cocaine: modification by Pavlovian conditioning. Pharmacol. Biochem. Behav. 15, 559.

Holloway, W.R., 1982. Caffeine: effects of acute and chronic exposure on the behavior of neonatal rats. Neurobehav. Toxicol. Teratol. 4, 21.

Hooks, M.S., Jones, G.H., Hemby, S.E., Justice, J.B., 1993. Environmental and pharmacological sensitization: effects of repeated administration of systemic or intra-nucleus accumbens cocaine. Psychopharmacology 111, 109.

Kalivas, P.W., Duffy, P., 1993. Time course of extracellular dopamine and behavioral sensitization to cocaine II. Dopamine axon terminals. J. Neurosci. 13, 266.

Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16, 223.

Kalivas, P.W., Sorg, B.A., Hooks, M.S., 1993. The pharmacology and neural circuitry of sensitization to psychostimulants. Behav. Pharmacol. 4, 315.

Kolta, M.G., Scalzo, F.M., Ali, S.F., Holson, R.R., 1990. Ontogeny of the enhanced behavioral response to amphetamine in amphetamine-pretreated rats. Psychopharmacology 100, 377.

Kostrzewa, R.M., 1995. Dopamine receptor supersensitivity. Neurosci. Biobehav. Rev. 19, 1.

Laviola, G., Dell'Omo, G., Alleva, E., Bignami, G., 1992. Ontogeny of cocaine hyperactivity and conditioned place preference in mice. Psychopharmacology 107, 221.

Le, W.D., Bostwick, R., Appel, S.H., 1992. Use of [^{3}H]-GBR12935 to measure dopaminergic nerve terminal growth into the developing rat striatum. Dev. Brain Res. 67, 375.

Lin-Chu, G., Robinson, T.E., Becker, J.B., 1985. Sensitization of rotational behavior produced by a single exposure to cocaine. Pharmacol. Biochem. Behav. 22, 901.

MacDougall, S.A., Crawford, C.A., Nonneman, A.J., 1992. Effects of irreversible dopamine receptor inactivation on locomotor activity and grooming in the 17- and 90-day-old rat. Psychopharmacology 106, 502.

MacDougall, S.A., Duke, M.A., Bolanos, C.A., Crawford, C.A., 1994. Ontogeny of behavioral sensitization in the rat: effect of direct and indirect dopamine agonists. Psychopharmacology 116, 483.

Marascuilo, L.A., Serlin, R.C., 1988. Statistical Methods for the Social and Behavioral Sciences. W.H. Freeman, New York, NY, p. 431.

Mattingly, B.A., Hart, T.C., Lim, K., Perkins, C., 1994. Selective antagonism of dopamine D_1 and D_2 receptors does not block the development of behavioral sensitization to cocaine. Psychopharmacology 114, 239.

Meyer, J.S., Yacht, A.C., 1993. Lack of behavioral sensitization to repeated cocaine administration from postnatal days 1 to 10. Int. J. Neurosci. 72, 107.

Pan, H.T., Menacherry, S., Justice, J.B., 1991. Differences in the pharmacokinetics of cocaine in naive and cocaine-experienced rats. J. Neurochem. 56, 1299.

Pettit, H.O., Pettit, A.J., 1994. Disposition of cocaine in blood and brain after a single pretreatment. Brain Res. 651, 261.

Post, R.M., Weiss, S.R.B., Pert, A., 1992. Sensitization and kindling effects of chronic cocaine. In: Lakoski, J.M., Galloway, M.P., White, F.J. (Eds.), Cocaine: Pharmacology, Physiology and Clinical Strategies. CRC Press, Boca Raton, FL, p. 115.

Rao, P.A., Molinoff, P.B., Joyce, J.N., 1991. Ontogeny of dopamine D_1 and D_2 receptor subtypes in rat basal ganglia: a quantitative autoradiographic study. Dev. Brain Res. 60, 161.

Rebec, G.V., Bashore, T.R., 1984. Critical issues in assessing the behavioral effects of amphetamine. Neurosci. Biobehav. Rev. 8, 153–159.

Scalzo, F.M., Holton, R.R., 1990. The ontogeny of behavioral sensitization to PCP and cocaine. Soc. Neurosci. Abstr. 16, 750.

Scalzo, F.M., Holton, R.R., 1992. The ontogeny of behavioral sensitization to phencyclidine. Neurotoxicol. Teratol. 14, 7.

Sobrian, S.K., Weltman, M., Pappas, B.A., 1975. Neonatal locomotor activity and long-term behavioral effects of *d*-amphetamine in the rat. Dev. Psychobiol. 8, 241.

Stewart, J., Badiani, A., 1993. Tolerance and sensitization to the behavioral effects of drugs. Behav. Pharmacol. 4, 289.

Tepper, J.M., Damlama, M., Trent, F., 1994. Postnatal changes in the distribution and morphology of rat substantia nigra dopaminergic neurons. Neuroscience 60, 469.

Terry, P., 1992. Differential effects of injection regimen on behavioral responses to cocaine. Pharmacol. Biochem. Behav. 41, 365.

Tsuchida, K., Ujike, H., Kanzaki, A., Fujiwa, Y., Akiyama, K., 1994. Ontogeny of enhanced striatal dopamine release in rats with methamphetamine-induced behavioral sensitization. Pharmacol. Biochem. Behav. 47, 161.

Tsuchida, K., Akiyama, K., Sakai, K., Ujike, K., Li, X., Kuroda, S., 1996. Ontogeny of striatal dopamine release in rats after acute administration of methamphetamine. Pharmacol. Biochem. Behav. 53, 575.

Ujike, H., Tsuchida, K., Akiyama, Y., Akiyama, K., Fujiwara, Y., Kuroda, S., 1995. Ontogeny of behavioral sensitization to cocaine. Pharmacol. Biochem. Behav. 50, 613.

Westerga, J., Gramsbergen, A., 1990. The development of locomotion in the rat. Dev. Brain Res. 57, 163.

Zahniser, N.R., Peris, J., 1992. Neurochemical mechanisms of cocaine-induced sensitization. In: Lakoski, J.M., Galloway, M.P., White, F.J. (Eds.), Cocaine: Pharmacology, Physiology and Clinical Strategies. CRC Press, Boca Raton, FL, p. 229.