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Ontogeny of Behavioral Sensitization to Cocaine

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UJIKE, H., K. TSUCHIDA, K. AKIYAMA, Y. FUJIWARA AND S. KURODA. *Ontogeny of behavioral sensitization to cocaine.* PHARMACOL BIOCHEM BEHAV **50**(4) 613-617, 1995.—The ontogeny of the behavioral effects of acute cocaine administration and behavioral sensitization to cocaine in rat pups was investigated. Acute behavior stimulating effects of cocaine were observed in pups as young as 7 postnatal days (PND) old, although they needed a higher dose of cocaine than adult rats to evoke the same motor effects. An adult dose-response curve pattern of stereotypy and locomotion to acute cocaine treatment was observed at PND 21, and of rearing at PND 28. Rats aged PND 7, 14, 21, 28, and 56 received repeated injections of saline or cocaine (15 mg/kg) twice a day for 5 consecutive days. After a 3-week period of abstinence, sensitization to a challenge dose of cocaine was assessed. Cocaine-induced stereotyped behavior was enhanced significantly only in rats in which cocaine pretreatment was initiated on PND 21, 28, and 56, but not earlier on PND 7 and 14. Adult female rats given repeated cocaine injections on PND 56-60 showed significantly greater sensitization than males, but no such sex difference was observed in pups given cocaine repeatedly on PND 21-25 or 28-32. These results show clearly that cocaine-induced behavioral sensitization in rats occurred only when subchronic cocaine administration was commenced on PND 21 or later.

Cocaine Behavioral sensitization Ontogeny Sexual difference D_1 and D_2 dopamine postsynaptic receptors

COCAINE is a psychostimulant agent that has caused serious social worldwide problems. Cocaine has a considerable abuse potential, and its abuse induces a psychosis in humans that resembles the paranoid type of schizophrenia, characterized by delusions, hallucinations, and stereotyped behavior. In laboratory animals, repeated cocaine administration has been found to induce progressive augmentation of locomotion, stereotyped sniffing, and head movements. This phenomenon, termed "behavioral sensitization," has been used as an animal model of human cocaine psychosis and schizophrenia (23, 24).

To elucidate the mechanisms of behavioral sensitization, we investigated the ontogenesis of sensitization in rats induced by methamphetamine, another potent psychostimulant, which also causes a schizophrenia-like psychosis in humans. We found that prenatal treatment of rats with methamphetamine did not induce a change in the sensitivity to a subsequent methamphetamine injection received in adulthood (5). However, a postnatal study revealed that pups receiving methamphetamine repeatedly after, but not before, a certain age developed sensitization to this agent (6,32). Similar results were obtained in an ontogenetic study of amphetamine-induced sensitization (16). One ontogenetic study of cocaine-induced sensitization during the late postnatal period was carried out, which showed that the sensitization intensity among the rats pretreated repeatedly with cocaine commenced on postnatal days (PND) 35, 49, and 91 did not differ (14). However, to our knowledge, no ontogenetic study of cocaine-induced sensitization during the earlier postnatal period has been carried out. Therefore, the aim of this study was to determine which stage of brain maturity must be reached for cocaine-induced sensitization to develop.

MATERIALS AND METHODS

The subjects were the offspring (both sexes) of Sprague-Dawley rats bred in our laboratory. All the animals were housed in a temperature- and humidity-controlled room maintained on a 12 L : 12 D cycle with lights on at 0800 h, and were allowed access to food and water ad lib. The pups were housed with their dams until PND 21, and then weaned. When co-

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caine treatment was started, any pup whose weight deviated from the average by more than ± 10% were excluded from the following experiments.

Experiment 1

Litters aged PND 7, 14, 21, 28, and 56 were divided randomly into four groups of five males and five females each. The pups in each group received a single intraperitoneal (IP) injection of sterile saline (2 ml/kg) or cocaine (7.5, 15, and 30 mg/kg dissolved in the same volume per kilogram of saline), and their induced behavior was analyzed as described subsequently.

Experiment 2

As described for Experiment 1, litters of each age were culled and divided into two groups. Five sets of groups, each composed of 10–12 pups of both sexes, received repeated IP injections of saline (2 ml/kg) or cocaine (15 mg/kg in the same volume per kilogram of saline) twice a day for 5 consecutive days (PND 7–11, 14–18, 21–25, 28–32, and 56–60), after which all pups underwent a 3-week period of abstinence followed by a single injection of cocaine (15 mg/kg). Thus, the cocaine challenge test was carried out on PND 32, 49, 56, 63, and 81, respectively.

Behavior Rating

The subjects were placed individually in an observation cage made of transparent plastic with dimensions 310 × 360 × 175 mm, and allowed to acclimatize for 1 h before drug treatment. Each animal was videotaped for 30 s every 5–15 min after the injection. Three types of behavior—activity and stereotypy; locomotion; and rearing—were scored by a single trained rater, who was unaware of the treatment and pretreatment conditions, according to the rating system of (33) as follows. Rearing was estimated in terms of the total duration when both forepaws were raised from the floor. Locomotion was scored as the number of times an animal moved from one corner of the cage to the other. Activity and stereotypy involving sniffing and repetitive head movement was rated using a score of 0–5: 0, asleep or still; 1, locomotion with normal exploration and normal pattern of sniffing and head movement; 2, increased rate of sniffing and head movement associated with hyperlocomotion and rearing; 3, discontinuous stereotyped sniffing and stereotyped up and down head movement with periodic locomotion activity; 4, almost continuous stereotyped sniffing and head movement, but sometimes interrupted by brief locomotion; 5, continuous and intense stereotyped sniffing and repetitive head movement at one location only. Statistical analyses for Experiments 1 and 2 were made by one-way ANOVA followed by multiple comparison test of Fisher's protected least significant difference test and two-way ANOVA, respectively. Differences at a probability level of 5% or less were considered to be significant.

RESULTS

Acute Motor Stimulant Effects of Cocaine in Pups of Various Postnatal Ages (Fig. 1)

There were no differences between the sexes at any age with respect to the intensity of saline- and cocaine-induced motor activity. Therefore, the following comparisons were made using the combined results of both sexes (n = 10/group). Rats as young as PND 7 showed activation of motor activity in response to acute cocaine treatment. Cocaine at 30 mg/kg, but not at lower doses, induced significantly more intense locomotion and activity and stereotypy in 7-PND pups than saline. In the 14-PND pups, 15 mg/kg cocaine also enhanced these types of behavior significantly, and 30 mg/kg enhanced

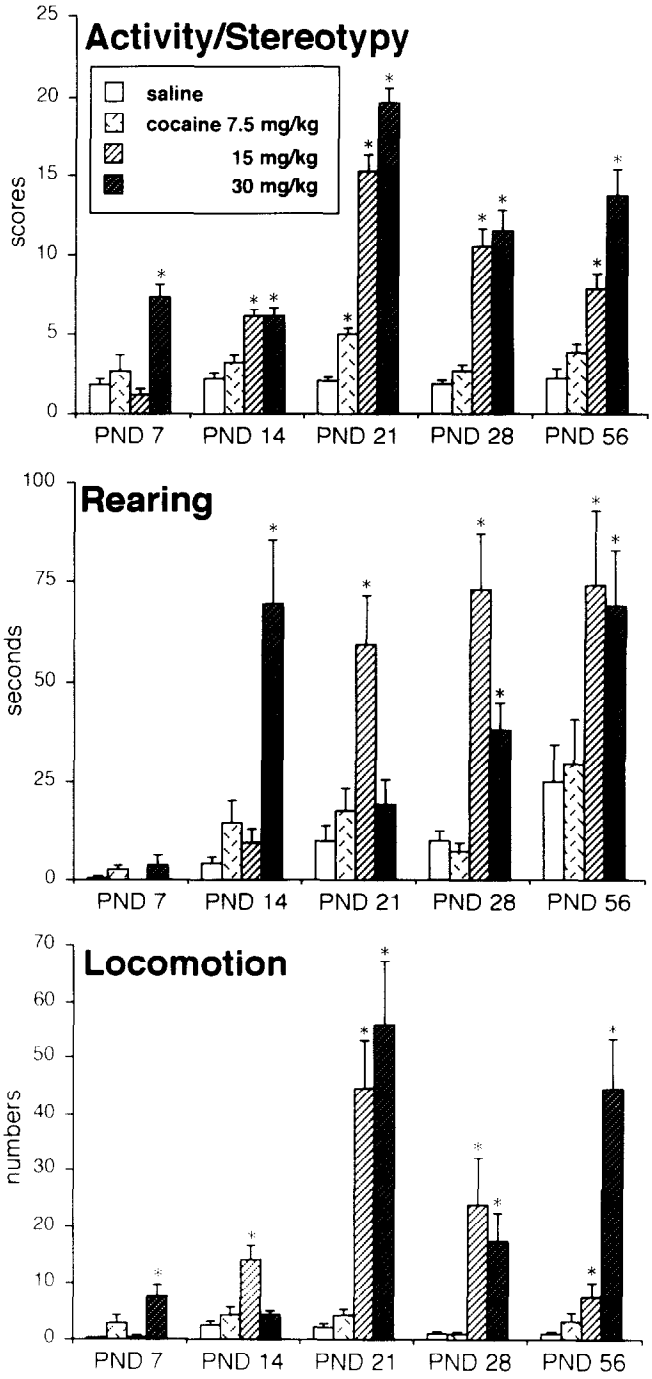

FIG. 1. Acute motor stimulant effects of cocaine in pups of various postnatal ages. Pups of each postnatal day (PND) received IP injection of saline or cocaine. Induced behavior of activity/stereotypy, rearing, and locomotion was assessed for 60 min. Each column shows the average and SEM of the summation of eight values obtained every 5–15 min after the injection. *Significant difference from saline analyzed by one-way ANOVA with Fisher's plsd test.

rearing behavior. However, the magnitude of the cocaine-induced enhancement was less in 7- and 14-PND pups than in adult rats given the same dose. The mature dose-response curve patterns of cocaine-enhanced activity and stereotypy and rearing were observed in rats treated starting on PND 21 and 28, respectively, whereas with cocaine-induced locomotion, the response intensity increased up to PND 21, and then decreased somewhat thereafter.

Ontogeny of Behavioral Sensitization to Cocaine (Fig. 2)

Adult rats that received repeated cocaine injections on PND 56–60 developed supersensitivity of activity and stereotypy and rearing to a challenge dose of cocaine given 3 weeks later [activity and stereotypy: $F(1, 144) = 23.58, p < 0.0001$; rearing: $F(1, 144) = 11.47, p < 0.009$]. However, the intensities of the three types of behavior in pups that received repeated cocaine treatment on PND 7–11 and in their corresponding controls did not differ when a challenge dose of cocaine was administered after 3 weeks of abstinence. The pups that received cocaine on PND 14–18 also showed no sensitization to a subsequent cocaine challenge. However, the pups that received cocaine on PND 21–25 showed significantly more intense activity and stereotypy, but not rearing or locomotion, after the cocaine challenge than their controls [activity and stereotypy; $F(1, 144) = 14.50, p < 0.0001$], and those given cocaine on PND 28–32 also showed sensitization development to cocaine in not only activity and stereotypy, but also locomotion and rearing [activity and stereotypy: $F(1, 176) = 52.20$, $p < 0.0001$; locomotion: $F(1, 176) = 10.17$, $p = 0.0017$; rearing: $F(1, 176) = 17.50, p < 0.0001$].

Effects of Sexual Difference on Ontogeny of Behavioral Sensitization to Cocaine (Fig. 3)

Concerning sexual difference, adult female rats showed significantly more intense sensitization of activity and stereotypy and locomotion than adult males [activity and stereotypy: $F(1, 64) = 17.12$, $p < 0.0001$; locomotion: $F(1, 64) = 10.19$, $p < 0.005$]. However, there were no sex differences between the intensity of sensitization to cocaine in pups that received it repeatedly on PND 21–25 and 28–32.

DISCUSSION

To our knowledge, this is the first demonstration that there is a turning point of age after which rat pups can be sensitized by repeated treatment with cocaine. Pups given cocaine repeatedly on PND 21–25 or 28–32 developed sensitization to cocaine, whereas those that received it earlier on PND 7–11 or 14–18 did not. The failure of cocaine-induced sensitization in younger pups did not result from an ineffectiveness of motor stimulation induced by acute cocaine administration, as this stimulated stereotyped behavior in pups as young as PND 7 despite their immature motor organs. Therefore, the induction process of sensitization to cocaine may only occur when a certain level of developmental maturity in the CNS has been reached.

In adult rats, cocaine-induced sensitization was subject to sex differences; females rats were sensitized more intensely than males. This phenomenon was also observed in rats with amphetamine-induced sensitization (27). However, pups of both sexes that received multiple cocaine pretreatment on PND 21–25 or 28–32 did not show any differences in the intensity of sensitivity to a subsequent cocaine challenge. This finding suggests that sex hormones secreted in adulthood may act as modulators or enhancers of cocaine-induced sensitization. In this study, female rats received daily injections of cocaine consecutively independent of the estrus cycle. An additional study is needed to determine which phase of the estrus cycle or which gonadal hormones may influence the development sensitization to cocaine.

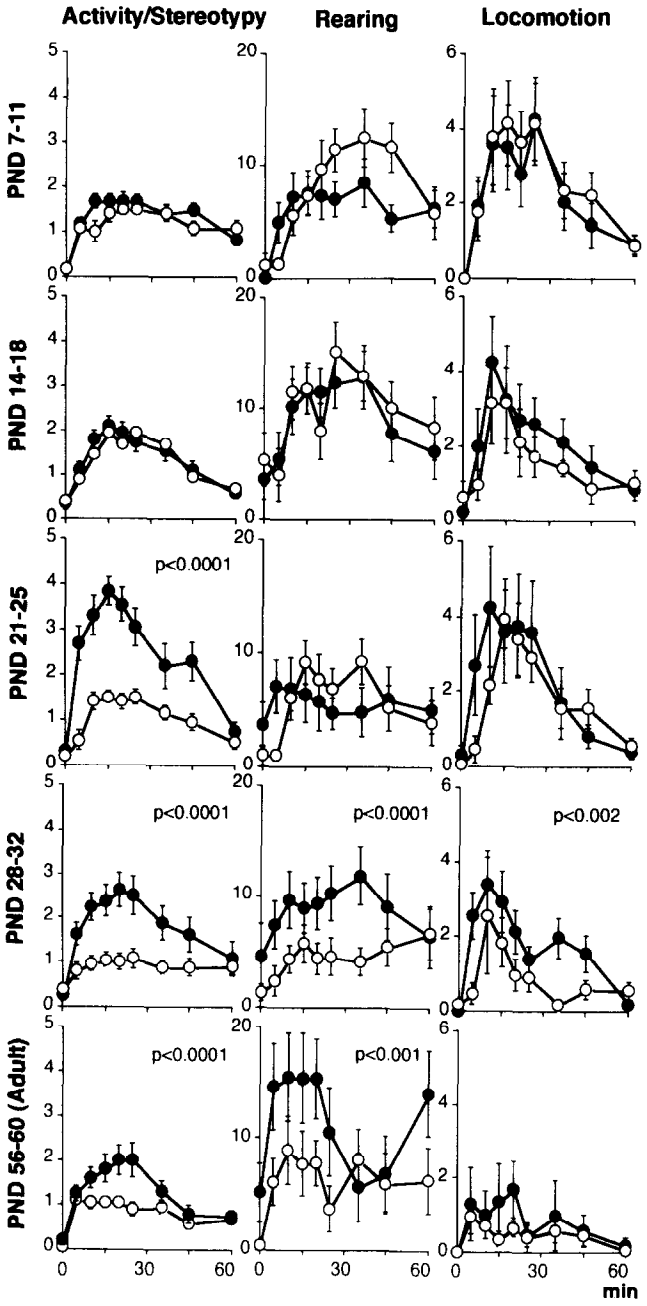

FIG. 2. Challenge tests with cocaine at dose of 15 mg/kg. Pups aged PND 7, 14, 21, 28, and 56 received injections of saline (oc) or cocaine (●) twice daily for consecutive 5 days. After a 3-week abstinence, all rats of groups received challenge doses of cocaine. See the text for F values of two-way ANOVA.

The results of this ontogenetic study of cocaine sensitization agree with those of our previous studies of methamphet-

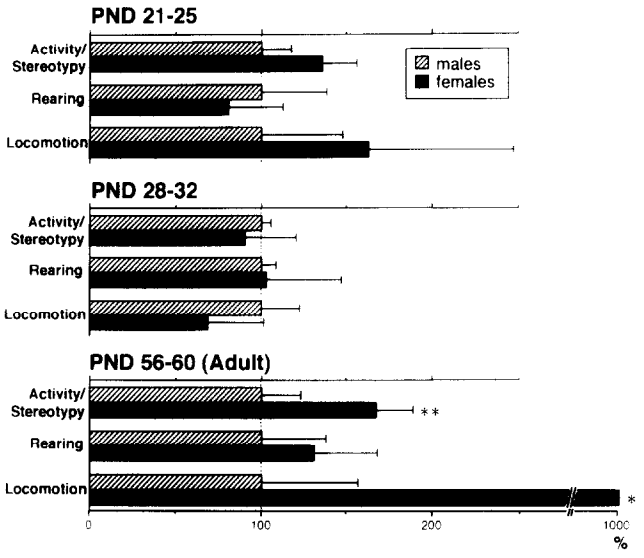

FIG. 3. Sex differences in the intensity of sensitization to cocaine. Each score of behavior observed after cocaine challenge in the rats pretreated with repeated cocaine during PND 21–25, 28–32, and 56–60 was summarized as described in Fig. 1. The data of females were shown as percentages of those of the corresponding males. $*F(1, 64) = 10.19$, $p < 0.005$; $**F(1, 64) = 17.12$, $p < 0.0001$ by two-way ANOVA.

amine-induced sensitization. We demonstrated that a certain level of brain developmental maturity must be reached in rats before behavioral sensitization to methamphetamine will develop, as the cocaine sensitization results of this study also have shown. Prenatal exposure to methamphetamine by treating the dam repeatedly with this agent did not induce sensitization in her offspring (5). In our postnatal experiment, pups received methamphetamine treatment for 5 consecutive days starting on PND 2, 7, 12, 17, 22, and 27 (6). The responses to a challenge dose of methamphetamine given on PND 35 showed that only pups treated with methamphetamine starting on PND 22 and 27, but not earlier, developed sensitization to it. However, in this study, all pup groups received the methamphetamine challenge dose at the same postnatal age. Therefore, those that showed sensitization to it were challenged after a shorter abstinence period than those that did not. This raises the possibility that a shorter duration of abstinence between the pretreatment session and challenge test may affect the development of methamphetamine-induced sensitization. However, this is unlikely, as we obtained identical results in our recent study, in which the methamphetamine challenge dose was administered after the same abstinence period of 3 weeks in all groups of pups (32). In this study, we also found that enhancement of psychostimulant-induced striatal dopamine release, one of the main neurochemical sensitization mechanisms, occurred only in pup groups given methamphetamine repeatedly starting on PND 21 or later. Kolta et al. (16) reported that pups given daily amphetamine injections from PND 49, but not PND 21, showed sensitization to a subsequent amphetamine challenge. This finding appears to differ somewhat from the findings of our methamphetamine and cocaine studies. However, their administration schedule differed from ours, and they did not examine pups at ages between PND 21 and 49. Thus, the exact age at which the pups became susceptible to amphetamine-induced sensitization is not clear. Therefore, we conclude that the sensitization induced by cocaine and methamphetamine, at least, demonstrate common ontogenetic characteristics. Brain maturity of at least 3 postnatal weeks must be reached before the psychostimulant-induced sensitization can occur.

Although several lines of evidence have indicated that many different neurotransmitter systems may be involved in the psychostimulant-induced sensitization mechanisms (10, 27), it is noteworthy that the central dopaminergic system is well developed during the early postnatal period in rats. Dopaminergic neurons and their receptors have been shown to be present in the middle embryonic period (2,11,26,28), but each component of the dopaminergic system develops at a different rate thereafter. The dopamine content and tyrosine hydroxylase activity, a rate-limiting dopamine synthesis enzyme, in the rat striatum increased linearly up to PND 30 or 60 (3,4,7, 12,20,22). A [^{3}H]-dopamine uptake study (15) and [^{3}H]-GBR 12783 and [^{3}H]-mazindol binding studies (1,25) showed that the dopamine reuptake site, the major site of action of cocaine, takes 40 or 60 days after birth to mature. Nigral D_2 dopamine receptors that act as autoreceptors on the soma of dopamine cells are already functionally mature at birth (19, 31), and presynaptic D_2 autoreceptors on the nigrostriatal projection terminals show full responses to dopamine receptor agonists as early as PND 4 or 14 (8,9,13,29). Accordingly, the time of maturity in these components of the dopamine system, and the dopamine content itself, its synthesis enzyme activity, uptake sites, or autoreceptors, are not coincide with an ontogenetic profile of cocaine-induced sensitization. However, postsynaptic dopamine receptors in the striatum seem to mature around the time of the turning point for susceptibility to cocaine sensitization. The densities of striatal D_2 dopamine receptors labeled by [^{3}H]-spiperone and [^{125}I]-iodobenzamide reach the adult level 3 weeks after birth in rats (17,18,25) and are accompanied by an increase in the striatal D_2 receptor mRNA level (30). Striatal D_1 receptors, which are located in the postsynaptic sites, also reach the adult level at this age (7,35), and striatal adenylate cyclase activity, which is mediated by activation of D_1 receptors, reaches maturity during this postnatal period (4,21). Therefore, the turning point for susceptibility to cocaine-induced sensitization coincides with the functional and numerical maturity of postsynaptic D_1 and D_2 dopamine receptors.

As an alternative explanation for the effects of ontogeny in the cocaine-induced behavioral sensitization, peripheral factors, such as the age-related changes in the activity of cocaine-metabolizing enzymes in blood and liver, should be considered. However, crucial roles of central D_1 and D_2 receptor for sensitization phenomenon induced by psychostimulants were also suggested by our previous study. We demonstrated previously that the D_1 receptor and D_2 receptor antagonists blocked methamphetamine-induced sensitization (34), which suggests that simultaneous activation of postsynaptic D_1 and D_2 receptors is essential for the induction of psychostimulant-induced sensitization. This finding is consistent with the hypothesis proposed by the findings of the ontogenetic study of cocaine-induced sensitization discussed here. Therefore, the neural plastic changes involved in psychostimulant-induced sensitization mechanisms may occur in sequence, starting from the activation of striatal D_1 and D_2 dopamine receptors, which mature at 3 weeks of age, in response to the repeated administration of psychostimulants.

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