Pharmacology Biochemistry and Behavior, Vol. 58, No. 4, pp. 1009–1013, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00311-0

Cocaine Impairs Maternal Nest Building in Pregnant Rats

VANYA QUIÑONES-JENAB, PHILIPPE BATEL, STEFAN D. SCHLUSSMAN, ANN HO AND MARY JEANNE KREEK

Laboratory of the Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10021

Received 12 September 1996; Revised 19 February 1997; Accepted 10 March 1997

QUIÑONES-JENAB, V., P. BATEL, S. D. SCHLUSSMAN, A. HO AND M. J. KREEK. *Cocaine impairs maternal nest building in pregnant rats.* PHARMACOL BIOCHEM BEHAV **58**(4) 1009–1013, 1997.—The present study investigated the onset of maternal nest building in pregnant Fischer rats following chronic repeated cocaine administration. Pregnant Fischer rats were injected with saline or cocaine, 15 mg/kg, three times daily at 1-h intervals for 10 days starting on gestation day 8. Cocaine-exposed females incorporated less material into their nests and built fewer fully completed circular nests than control animals. The overall quality of the nest in cocaine exposed dams was significantly lower than that of control animals. Furthermore, cocaine exposed dams gained less weight than control females. However, no difference in number of pups, weight, or length of pups was observed between groups. Thus, it seems that cocaine disrupts the interest and skill in nest building of pregnant rats. © 1997 Elsevier Science Inc.

"Binge" pattern cocaine Pregnancy Drug abuse Maternal behaviors Cocaine

THE growing use of cocaine among pregnant women has become an issue of great concern because a substantial population of infants are exposed gestationally to cocaine (10). Recent reports of the prevalence of cocaine use in the United States of America during pregnancy range from 10–17% (8). Maternal cocaine use during pregnancy is associated with medical and life-style characteristics detrimental to fetal and infant development (10). In humans, prenatal cocaine exposure has been reported to be associated with a variety of abnormal symptoms ranging from genitourinary tract malformations and intrauterine growth retardation, to neurological alterations such as elevated startle response, tremor, rigidity, seizure, microcephaly, learning disabilities, and behavioral disorders (4–6,10,14). Cocaine use may increase up to threefold the relative risk for anomalies in cocaine exposed fetuses vs. control perinates (it has been reported that 15.7% cocaine-exposed perinates exhibit anomalies vs. 5.4% perinates without known prenatal cocaine exposure) (9). Cocaine use during pregnancy has also been reported to significantly contribute to the risk of placental abruption, preterm delivery, low birth weight, stillbirth, and other defects (10). Most of the reported clinical studies focus on the physiological and behavioral consequences of cocaine abuse in offspring. Little is known regarding the effects of cocaine on maternal behavior or on possible neuroendocrine regulation in female humans and animals, especially during pregnancy.

Kinsley et al. have proposed that prenatally cocaine-exposed children may face poor maternal care, in combination with teratological effects of gestational cocaine exposure; thus, these offspring face a double jeopardy (11). This is also true for rats, where cocaine exposure significantly reduces maternal behaviors (11,20,23). Rats exposed to cocaine during pregnancy are less attentive to their young, spending significantly less time in their nest, with increased latencies to engage in maternal behaviors, resulting in reduced or eliminated maternal responsiveness (11).

Maternal behaviors can be defined as the species-typical activities in which the mother engages, to insure the survival and optimize the development of her offspring (13). During pregnancy, rodents engage in nest-building behaviors, which continue at a high level throughout lactation (13). A nest provides a place in which to bear and raise the young (2,21). Nest

Requests for reprints should be addressed to Vanya Quiñones-Jenab, Laboratory of the Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

building is instrumental in the successful rearing of young following parturition (3). Because cocaine exposure has been shown to affect the onset and maintenance of maternal behaviors in lactating rats (11), we wished to determine whether and to what extent maternal behaviors are affected earlier, during gestation, where little is known. Thus, in the present study we examined the effects of cocaine exposure during gestation on the nest-building behaviors of the pregnant rat.

GENERAL METHOD

Animals

Female Fischer rats purchased from Charles River were housed in an acoustically controlled stress-minimized facility in single-animal cages with free access to food and water, and maintained on a 12-h light–dark cycle (lights on at 0930 h). Two weeks after adjustment to the animal facility the females were time mated. The day that the sperm plug was found was defined as day 0 of pregnancy. The animals' cages were changed once a week before the first injection of the day.

Drug Administration

Twenty-four animals were randomly assigned to two groups (matched according to plug day). However, one animal in the cocaine group was found not to be pregnant, resulting in 11 cocaine-treated animals and 12 saline-treated animals. On gestation day 8 to 17, animals received three daily intraperitoneal injections of 0.9% saline (1 mg/kg of body weight) or cocaine (15 mg/kg of body weight dissolved in 0.9% saline at a concentration of 15 mg/ml), at 1000, 1100, and 1200 h. This dosing schedule was chosen to mimic the manner in which cocaine is often self-administrated by humans both in terms of temporal pattern and in relation to circadian rhythm (1). Because rats are nocturnal, the time of the injections approximates early evening hours for humans (1).

Assessment of Nesting Behavior

From gestation day 0 to 8 the animals were allowed to build nests ad lib. At 1000 h, before the first injection of the day, the nest quality was scored. The nest quality was rated on a 5-point scale ranging from 0 to 4, modified from Lisk et al. (12). A score of 0 was given when no nest was built by the female. A score of 1 was given when nesting material was present in a corner of the cage, but no organized nest was built. A score of 2 was given when some kind of organization of nesting material, such as a semicircular organization, was present in a corner of a cage, but no walls or more complex structure were present. A score of 3 was given when a complete circular or semicircular nest with walls was built, and a score of 4 was given to a full nest with tall walls.

The quality of the nest was recorded before daily drug or saline administration. On the first, fifth, and ninth day of treatment, at 1000 h the nests were removed from the cage. After the third injection of the day, 25 g of nesting material (compressed cotton) was put on the top of the cage. The animals were then observed for a period of 30 min. The time until the animal demonstrated interest in the nesting material and the time until the animal brought the nesting material to a corner of the cage were each recorded.

The next morning the quality of the nest was scored and the amount of nesting material utilized was recorded. Because major physiological changes occur after gestation day 17, such as the increase of estrogen and oxytocin levels that occurs later in the third trimester when dams are preparing for parturition, gestation day 17 was chosen for sacrifice by decapitation after exposure to CO_2. The number of embryos, their size, and the number of resorptions were recorded.

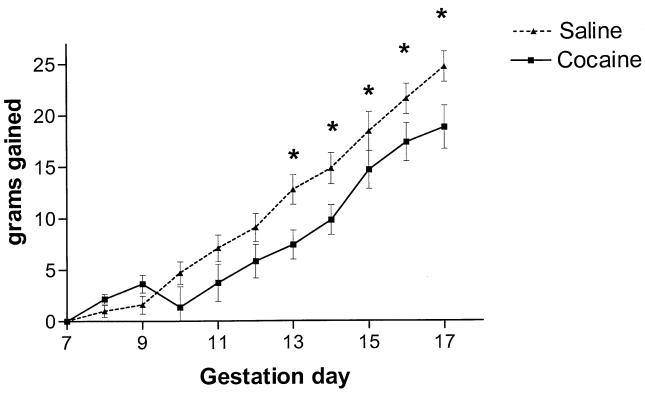

FIG. 1. Mean (±SEM) daily weight gain from gestation day 7 (1 day before treatment) to gestation day 17 of cocaine- and saline-treated dams. Cocaine administration affected body weight gain in pregnant animals. *Represents a significant difference determined by ANOVA followed by Newman–Keuls post hoc tests, $p < 0.05$.

Statistics

A one-way analysis of variance (ANOVA) with repeated measures followed by Newman–Keuls post hoc tests was used to determine the significance of differences between treatment groups in body weight. To examine the effect of cocaine on the quality of maternal nest building, a nonparametric approach was used in the analysis of the daily behavioral ratings: A Kruskal–Wallis ANOVA by ranks was used to determine the significance of differences between the groups on each day of scoring. To look at the effect over the entire treatment period (from the day before starting treatment to the 10th day of "binge" pattern cocaine administration), the median value of the 11 behavioral scale scores was used in a Kruskal–Wallis ANOVA. Finally, to examine the significance of the differences between groups in the nest-building parameters of grams of nesting material used, interest in nesting and time to bring material to cage corner measured in minutes, with

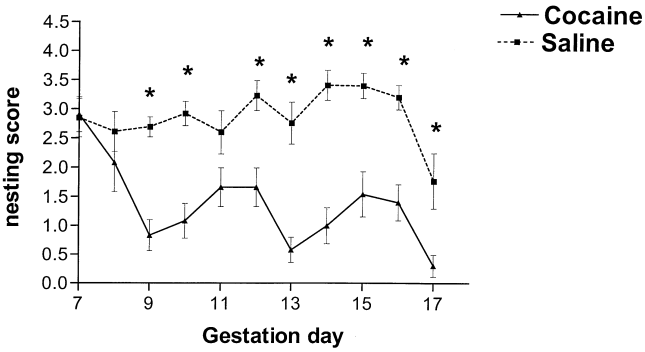

FIG. 2. Mean (±SEM) daily nesting scores from gestation day 7 (1 day before treatment) to gestation day 17 of cocaine- and saline-treated dams. Cocaine administration affected nest-building behavior in pregnant females. *Represents a significantly lower score, Kruskal–Wallis ANOVA by ranks, $p < 0.05$.

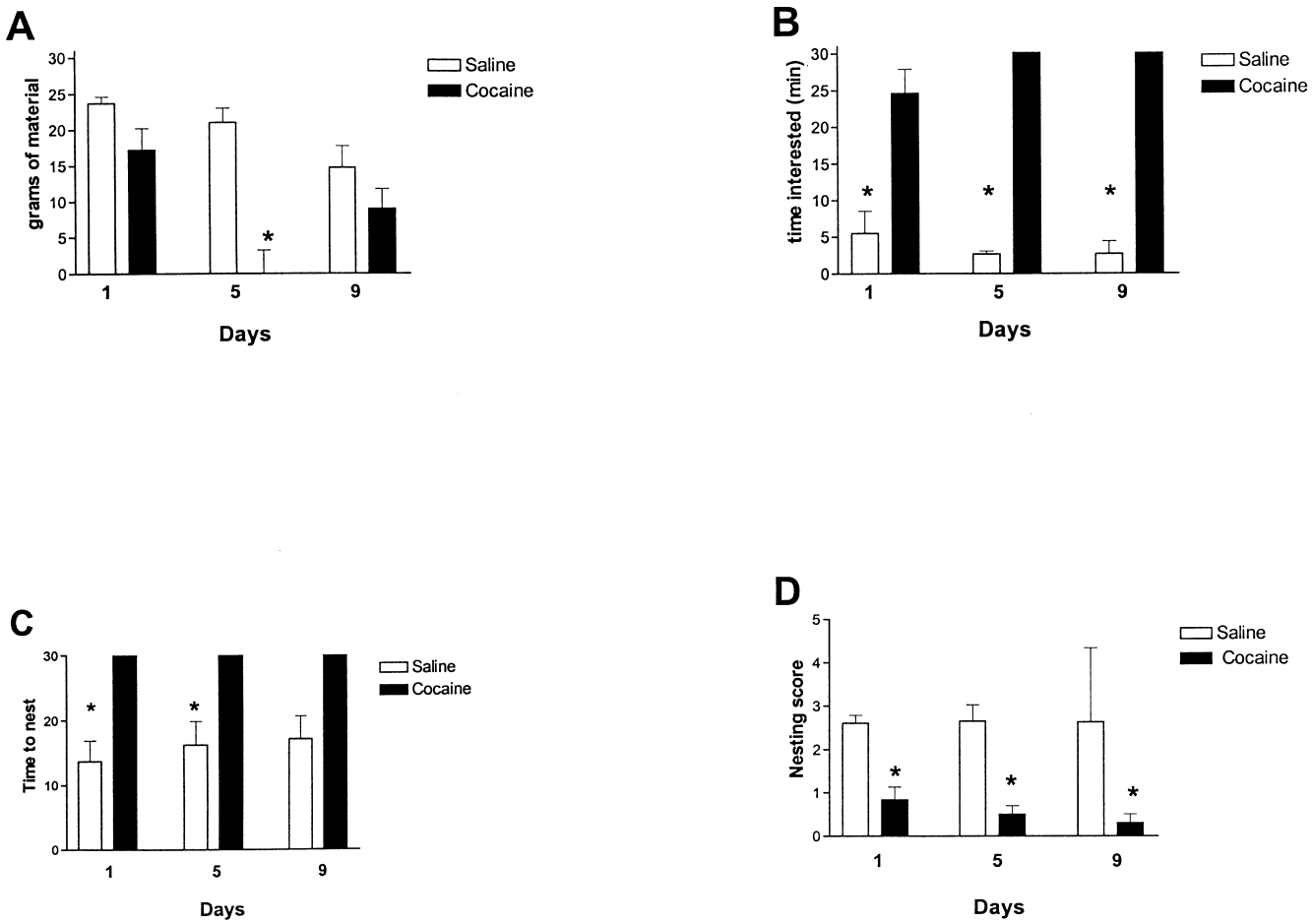

FIG. 3. Effect of binge cocaine administration on the nesting activity of dams during treatment days 1, 5, and 9. Mean amount of cotton pulled for nest building (g) (A), time to show interest (latency in minutes) (B), time to start building the nest (in minutes) (C), and nesting score (D) on first, fifth and ninth day of treatment. (Values shown as mean ± SEM, *represents significant differences between groups by Mann–Whitney U, $p < 0.05$).

skewed distributions, the Mann–Whitney *U*-test was used. Significance in all cases was considered to be $p < 0.05$.

RESULTS

The overall weight gain of cocaine-treated and control dams is shown in Fig. 1. The pattern of weight gain of the two groups over the course of the study was significantly different [the group by day interaction $F(1, 198) = 4.15$, $p < 0.0001$]. After day 3 when the cocaine-treated dams failed to gain weight, the weight gain between the groups was almost parallel. Nevertheless, the cumulative effect was such that after the sixth day of treatment (gestation day 13), cocaine-treated dams had gained significantly less weight than control animals (Newman–Keuls post hoc tests, $p < 0.02$ or less).

The mean daily nesting scores of both groups from gestational day 7 to 17 are shown in Fig. 2. The scores of the saline-treated animals were higher than those of the cocaine-treated dams from day 2 of treatment (gestational day 9) through day 10 of treatment (gestational day 17), as reflected in a significant difference (Kruskal–Wallis ANOVA by ranks) on each day save the fourth (gestational day 11). An analysis of the median nesting score for each dam across the 11 days of treatment also showed a significant difference between groups (Kruskal–Wallis $H = 15.17$, $p = 0.0001$).

Cocaine-treated dams incorporated less nesting material into their nests on day 5 of treatment (gestational day 12) than control dams (Mann–Whitney $U = 34.5$, $p < 0.02$), as can be seen in Fig. 3A. Control animals had significantly shorter latencies to demonstrate the initial interest in nesting material (determined as the time until the pregnant dam investigates the nesting material), as well as the time until that the animal brings nesting material to a corner of the cage (Mann–Whitney *U*-tests, $p < 0.05$ or less as shown in Fig. 3B and C, respectively). Furthermore, the quality of nests built by cocaine-treated dams was significantly decreased during these test days (Kruskal–Wallis ANOVAs by rank, $p < 0.05$ or lower), as may be seen in Fig. 3D. No differences between groups in the number or size of fetuses were observed (Fig. 4).

DISCUSSION

We demonstrated that cocaine can impair the ability of pregnant females to build a nest. Cocaine-exposed pregnant rats took longer to show interest in nesting material and longer to initiate their nesting activities, utilized less material in their nest, and their nests were of poor quality than control dams.

During postpartum periods, Zimmerberg and Gray (23) observed that a low dose of cocaine (5 mg/kg/day, intraperitoneal) had no effect on nest-building behaviors of dams. How-

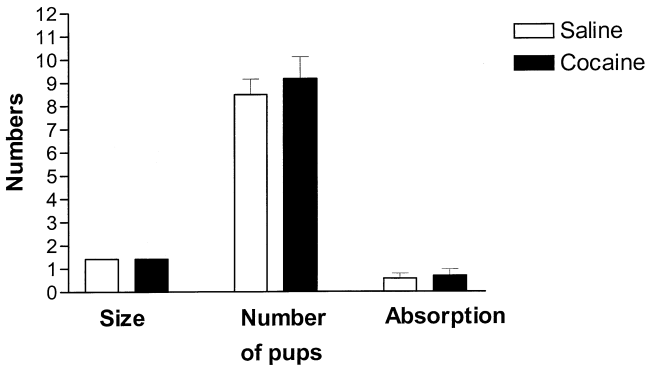

FIG. 4. Effect of binge cocaine administration on the number and size (in cm) of pups in litters and number of fetal resorption in pregnant female animals.

ever, a recent study by Vernotica et al. (20) demonstrated that higher doses of cocaine (20 and 40 mg/kg/day, subcutaneous, in a single dose) significantly impaired nest-building behavior in dams during postpartum. Furthermore, Vernotica et al. (20) demonstrated that the deficit in nest building persisted for as long as 4 h following injection. Thus, the route, dose, and mode of cocaine administration may have an effect on the nest-building behaviors. It is feasible that higher doses of cocaine such as those used by us and Vernotica et al. (20) than those used by Zimmerberg and Gray (23) have a significant effect on the disposition of the females to build nests both during gestation (shown here) and after parturition (20).

In this study we demonstrated that 22 h after "binge" pattern cocaine administration (15 mg/kg, three times/day, intraperitoneal) the amount of nesting material incorporated into the nest was reduced and the quality of the nest built was impaired in pregnant dams. It has been shown that the locomotor effects of cocaine in males Fischer rats (using the same cocaine administration paradigm) are no longer evident four hours after the last injection of the day (19). Thus, the effect of cocaine on the nest qualilty reported here is not likely to be due to locomotor effects of cocaine.

The impairment in nest-building behavior after cocaine exposure could be mediated by changes in the cascade of hormonal and neurochemical factors that regulate the stimulation and/or maintenance of the maternal behaviors. Construction of the maternal nest has been reported to be regulated in part by ovarian and brain hormones (3,15,16,21). These hormones include oxytocin, prolactin, progesterone, and estrogen, each of which has been shown to interact with opioids (7,18). Although no neurochemical assessments were conducted in these experiments, it is provocative to speculate that the effects of cocaine on nest-building behaviors are mediated at those neurochemical levels. Further studies are needed to examine these relationships.

A decrease in nesting behavior on days 16 and 17 of gestation has been previously shown in pregnant mice (2). In rats, progesterone decreases on gestation day 17 (13). Thus, the sharp decrease we observed in the nesting behavior both in cocaine and saline rats may be in part regulated by levels of progesterone and/or its receptor.

Although we hypothesizes that gestational exposure to cocaine can result in differential regulation of neuroendocrine mechanisms, other alternative mechanisms, such as an increase in locomotor activation, stereotypic behaviors, stress responses (each of which has been found to be affected in male Fischer rats using "binge" pattern cocaine administration (17,19,22)), and/or thermoregulatory mechanisms, could contribute to the effects of cocaine on the nest-building activity of the dams. The role of each of these mechanisms in nest-building behaviors remains to be determined.

ACKNOWLEDGEMENTS

We appreciate the help of Dr. Sonoko Ogawa in the design of these experiments and her input on the discussion of the results. This work was supported by NIH-NIDA P50-DA05130 (M. J. K.) and NIH-NIDA K05-DA0049 (M. J. K.).

REFERENCES

1. Branch, A. D.; Unterwald, E. M.; Lee, S. E.; Kreek, M. J.: Quantitation of preproenkephalin mRNA levels in brain regions from male Fischer rats following chronic cocaine treatment using a recently developed solution hybridization procedure. Mol. Brain Res. 14:231–238; 1992.
2. Broida, J.; Svare, B.: Mice: Progesterone and the regulation of strain differences in pregnancy-induced nest building. Behav. Neurosci. 97:994–1004; 1983.
3. Broida, J.; Svare, J.: Strain typical patterns of pregnancy induced nest building in mice: Maternal and experiential influences. Physiol. Behav. 25:153–157; 1982.
4. Chasnoff, I. J.; Burns, W. J.; Schnoll, S. H.; Burns, K. A.: Cocaine use in pregnancy. N. Engl. J. Med. 313:666–669; 1985.
5. Chasnoff, I. J.; Griffins, D. R.; Burns, K. A.; Burns, W. J.: Cocaine use in pregnancy: Perinatal morbidity and mortality. Neurotoxicol. Teratol. 9:291–293; 1987.
6. Chasnoff, I. J.; Griffins, D. R.; MacGregor, S.; Dirkes, K.; Burns, K. A.: Temporal patterns of cocaine use in pregnancy. JAMA 261:1741–1744; 1989.
7. Douglas, A. J.; Dye, S.; Leng, G.; Russell, J. A.; Bicknell, R. J.: Endogenous opioid regulation of oxytocin secretion through pregnancy in the rat. J. Neuroendocrinol. 5:307–314; 1993.
8. Frank, D.; Zucherman, B. S.; Amaro, H.; Aboaqye, K.; Barchnaer, H.; Cabral, H.; Fried, L.; Hingson, R.; Kayne, K.; Levenson, S. M.; et al.: Cocaine use during pregnancy: Prevalence and correlates. Pediatrics 82:888–895; 1988.
9. Hume, R. F.; Gingars, J. O.; Martin, L. S.; Hertzberg, B. S.; O'Donnell, K.; Killam, A. P.: Ultrasound diagnosis of fetal anomalies associated with in utero cocaine exposure: Further support from cocaine-induced vascular disruption teratogenesis. Fetal Diagn. Ther. 9:239–245; 1994.
10. Kain, S. N.; Rimar, S.; Barash, P. G.: Cocaine abuse in the parturient and effects on the fetus and neonate. Anesth. Analgesia 77:835–845; 1993.
11. Kinsley, G. H.; Turco, D.; Bauer, A.; Beverly, M.; Wellman, J.; Graham, A. L.: Cocaine alters the onset and maintenance of maternal behavior in lactating rats. Pharmacol. Biochem. Behav. 47:857–864; 1994.
12. Lisk, R. B.; Pertlow, R. A.; Friedman, S. M.: Hormonal stimulation necessary for elicitation of maternal nest-building in the mouse. Anim. Behav. 17:730–737; 1969.
13. Numan, M.; Knobil, E.; Neil, J. D., eds.: The physiology of reproduction. New York: Raven Press; 1988:569–645.
14. Ryan, L.; Ehrlich, S.; Finnegan, L.: Cocaine abuse in pregnancy: Effects on the fetus and newborn. Neurotoxicol. Teratol. 9:295–299; 1987.
15. Schneider, J. E.; Lynch, C. B.: Investigation of a common physiological mechanism underlying progesterone-induced and mater-

nal nesting in mice, *Mus musculus*. J. Comp. Physiol. Psychol. 98:165–176; 1984.
16. Schneider, J. E.; Lynch, C. B.; Possidente, B.; Hegmann, J. P.: Genetic association between progesterone-induced and maternal nesting in mice. Physiol. Behav. 29:97–105; 1982.
17. Spangler, R.; Zhou, Y.; Schlussman, S. D.; Ho, A.; Kreek, M. J.: Behavioral stereotypies induced by "binge" cocaine administration are independent of drug-induced increases in corticosterone levels. Behav. Brain Res. 86:201–204; 1997.
18. Terenius, L.: Opioid peptides, pain, and stress. Prog. Brain Res. 375:383; 1992.
19. Unterwald, E. M.; Ho, A.; Rubenfeld, J. M.; Kreek, M. J.: Time course of the development of behavioral sensitization and dopamine receptor upregulation during binge cocaine administration. J. Pharmacol. Exp. Ther. 270:1387–1395; 1994.
20. Vernotica, E. M.; Lisciotto, C. A.; Rosenblatt, J. S.; Morrell, J. I.: Cocaine transiently impairs maternal behavior in the rat. Behav. Neurosci. 110:315–323; 1996.
21. Voci, V. E.; Carlson, N. R.: Enhancement of maternal behavior and nest building following systemic and diencephalic administration of prolactin and progesterone in the mouse. J. Comp. Physiol. Psychol. 83:388–393; 1993.
22. Zhou, Y.; Spangler, R.; LaForge, S.; Maggos, C.; Ho, A.; Kreek, M. J.: Corticotropin-releasing factor and type 1 corticotropin-releasing factor receptor messenger RNAs in rat brain and pituitary during "binge" pattern cocaine administration and chronic withdrawal. J. Pharmacol. Exp. Ther. 279:351–358; 1996.
23. Zimmerberg, B.; Gray, M. S.: The effects of cocaine on maternal behaviors in the rat. Physiol. Behav. 52:379–384; 1992.