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Opiate Withdrawal in the Fetal Rat: A Behavioral Profile

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JONES, K. L. AND G. A. BARR. *Opiate withdrawal in the fetal rat: a behavioral profile.* PHARMACOL BIOCHEM BEHAV **66**(2) 419–424, 2000.—Offspring of women exposed to opiate drugs such as heroin and methadone during pregnancy have a high incidence of morbidity and mortality. Infants also show opiate withdrawal. In this study, we examined the behavioral effects of precipitated withdrawal in morphine-dependent fetal rats at gestational day (GD) 20. The dam was implanted on GD 14 with a pellet containing 75.0 mg of morphine. On GD 20, the dam underwent chemoyelotomy at L1/L2. The uterine horns were externalized and four subject fetuses were selected for behavioral observation, two from each uterine horn. The fetus was then injected subcutaneously with either saline or naltrexone (1.0 mg/kg) and the behaviors of the fetus recorded every 15 sec for 20 min. The results show that naltrexone injected fetuses that had been chronically exposed to morphine demonstrated increased limb and mouth movements, face wiping, and body curls, and spent less time quiet as compared with control fetuses. These results indicate that a morphine withdrawal-like syndrome occurs in the fetal rat. © 2000 Elsevier Science Inc.

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EXPOSURE to opiate drugs such as heroin and morphine during pregnancy has been associated with a variety of adverse effects that range from premature birth to fetal and neonatal death, chromosomal aberrations, and decreased birth weights and head circumferences (4). Because opiates readily cross the placental barrier, these deleterious effects may be in part a result of the placental transport of the drug. Because one common characteristic observed in the pattern of drug use among pregnant women is that of repeated exposure and withdrawal, some of these effects may in fact be a result of the fetus experiencing withdrawal from the drug *in utero*.

A marked and persistent neonatal complication associated with maternal opiate exposure is the period of withdrawal that begins within 1 to 3 days of birth (9). In the human infant, the most common withdrawal symptoms include respiratory and gastrointestinal dysfunction, yawning, sneezing, and tearing, frantic sucking of fists, an inconsolable high-pitched cry, restlessness and irritability, and difficulty with feeding (8). Unfortunately, interpreting these data in humans is difficult because the drug-dependent woman is predisposed to a whole host of maternal complications. For example, a majority of the opiate addicts who become pregnant are of a low socioeconomic status and have a history of multiple drug abuse often paralleled with mental or medical illness (10). Many of these women get inadequate prenatal care and are poorly nourished.

In order to examine the effects of prenatal opiate exposure in the absence of maternal complications we must turn to the animal model. In the adult rat, withdrawal of opiates or administration of opiate antagonists in opiate-exposed animals precipitates a withdrawal syndrome. This syndrome is characterized by a variety of vegetative and motoric signs including weight loss, decreased consumption of food and water, diarrhea, eye twitching, rhinnorrhea, lacrimation, penile erection/

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ejaculation, increased sensitivity to touch, hostility on handling, teeth chattering, increased exploring, jumping, and wet dog shakes. Although the withdrawal syndrome in the adult animal is well defined, the available data describing the withdrawal syndrome in the developing animal are limited. In the rat, prenatal opiate exposure has effects parallel to those in the human, such as increased fetal and neonatal death and lower birth weights (9,15,19). Although early studies examining the neonatal withdrawal syndrome in the rat report that adult-like withdrawal does not occur until well after weaning in the rat (7), a neonatal withdrawal syndrome characterized by increased levels of ultrasonic vocalizations and specific behaviors appropriate to the age of the animal does occur (2,11,24–26). Morphine-treated rat pups, as young as 7 days of age, tested with naltrexone show increased rolling, stretching, and head and paw movements. The existence of a fetal withdrawal syndrome in the rat has been suggested based on the effects of morphine and naltrexone on the spontaneous activity in rat fetuses. Kirby (12,13) found that morphine caused a depression of activity in fetuses from the 15th through 21st day of gestation that was reversed by naloxone. However, a detailed behavioral profile of opiate withdrawal in the fetal animal has not been described. In an effort to describe behaviors that may reflect opiate withdrawal in utero, we precipitated withdrawal in morphine-exposed GD 20 fetuses (Gestation Day 20) and measured the resultant changes in behavior.

METHOD

Subjects

The subjects were fetuses from Long-Evans hooded rats mated in our colony. Thirty-three fetuses from nine litters were used. In six litters, 4 fetuses were used and from 3 litters, 3 fetuses were used. The parent rats were housed in plastic tubs in a colony room maintained at 22° to 24°C on a 12-h light-dark photocycle with light onset at 0700 h. Every morning, females were checked for the presence of sperm in the vaginal smears. Once sperm were detected, the male was separated from the female and that day was termed gestation day 0 (GD 0). All studies followed NIH guidelines and were approved by the Hunter College IACUC.

Treating the Dam

Drugs were delivered to the fetus indirectly through the dam. On gestation day 14, the dam was anesthetized with isofluorane and implanted with a 75-mg morphine pellet (generously supplied by NIDA), a standard technique used to produce opioid dependence in laboratory animals (1,5,27). Controls were dams that underwent the implant procedure but received no pellet.

Surgical Preparation

On GD 20 under the ultra short-acting anesthesia isofluorane, each female underwent chemomyelotomy. Ethanol (100%, 100 μL, delivered at room temperature) into the spinal cord between the first and second lumbar vertebrae (20). This produces an irreversible spinal anesthesia posterior to the site of injection rendering the female completely unresponsive to stimulation in the area of the hindquarters. Chemomyelotomy was chosen over spinal transection for its consistency between preparations, its comparability to recent studies of fetal behavior, and its lack of effect on the behavior of the fetus (20). Following the chemomyelotomy, the female was gently restrained in a holding apparatus, the uterus was exteriorized through a midline laparotomy, and her uterus and hindquarters were immersed in a temperature-controlled (37.5°C) bath containing isotonic saline. The number of fetuses in each uterine horn was counted. The mother and fetuses quietly recovered from the anesthesia and were acclimated to the water bath for 20 min before behavioral observations began.

Precipitation of Withdrawal (GD 20)

Following acclimation to the water bath, four subject fetuses were then selected for behavioral observation, two from each uterine horn. The first of the four subject fetuses was prepared for direct observation by the delivery of the subject fetus through a 10- to 15-mm incision in the uterine wall, maintaining the placental-uterine attachment intact, with removal of the amniotic membranes from around the fetus (24). The average weights of the fetuses were determined in preliminary studies. Two subject fetuses received an injection of naltrexone (approximately 1 mg/kg) and two received an injection of saline. The observer was blind to the treatment condition. The behavior of the fetus was then observed. At the end of each observation period the fetus was anesthetized with an intraperitoneal (IP) injection of sodium pentobarbital (10 mg/kg) to prevent interference with the next observed fetus. Although it is possible that the drug injections could spread to other fetuses via placental transport, dilution factors would lower drug concentrations. Furthermore, we observed no noticeable change in the behaviors of fetuses of either uterine horn following each observation. This suggests that there is negligible exchange of injected drugs among fetuses within each uterine horn during the observational period.

Behavioral Observations

Following the injection of naltrexone or saline, fetal behavior was scan sampled every 15 sec for a total of 10 min using a behavioral checklist (Table 1). This checklist was developed by integrating in utero behaviors outlined in previous descriptions of spontaneous behaviors that occur in the fetal rat with withdrawal behaviors of the infant and adult (21). This procedure required the cooperation of two people. One person, who was blind to the treatment condition verbally called out the behavior that the fetus was engaging in at each

TABLE 1
BEHAVIORAL DEFINITIONS

Behavior	Definition
Body curls	Ventral or lateral flexion of the trunk
Body twitch	Brief spasms along the flank of the fetus
Face wiping	Wiping of both forelimbs across the face
Foreleg movements	Flexion, extension or rotation of one or both hindlegs
Head movements	Ventral, dorsal, or lateral rotary motion of the head
Hindleg movements	Flexion or extension of one or both hindlegs
Mouth movements	Opening and closing the mouth
Quiet	Sedated appearance with no movement
Stretch	Extension or dorsal flexion of the trunk resulting in an apparent lengthening of the body

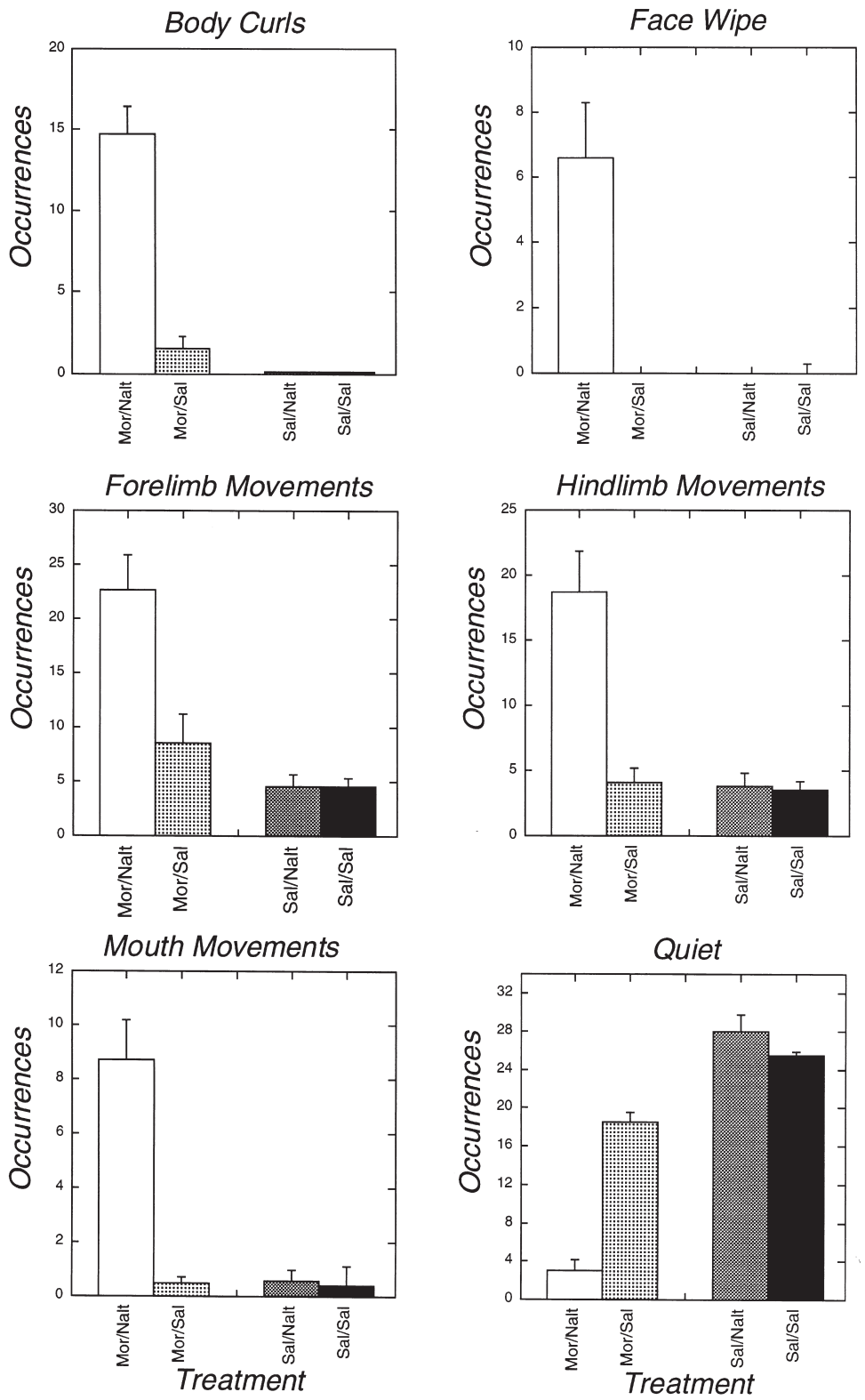
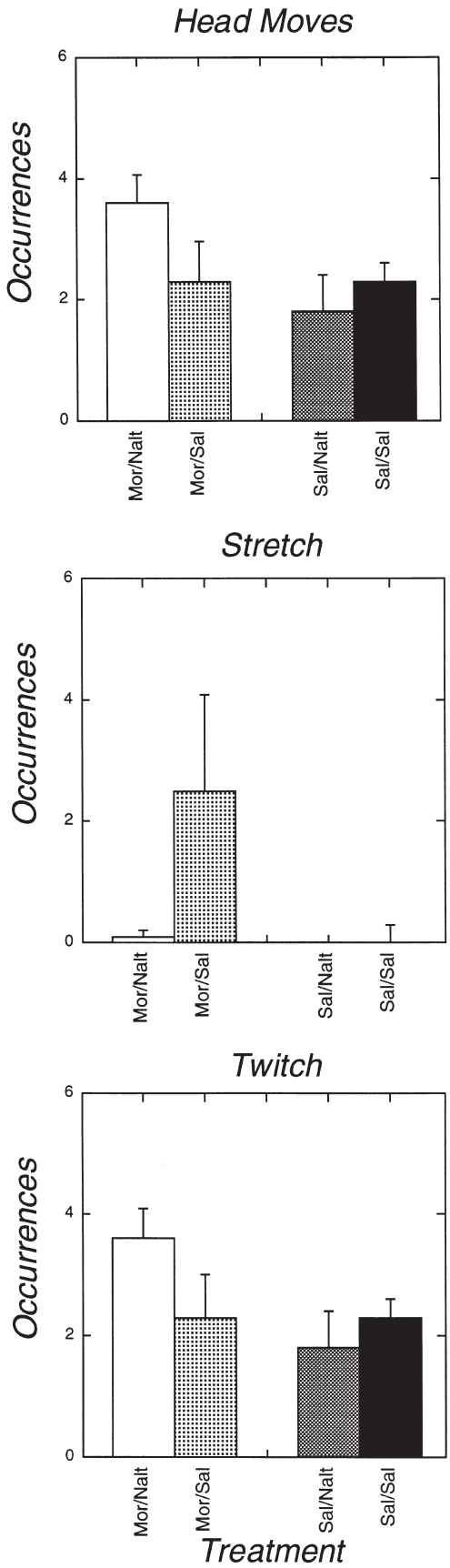


FIG. 1. Fetal withdrawal behaviors. These data are the mean number of occurrences ± one SEM of fetal behaviors. Fetuses were exposed to no treatment or chronic morphine exposure. At GD20 they were injected with saline or naltrexone and observed every 15 sec for 10 min. A significant interaction between acute treatment and chronic treatment defined withdrawal. As can be seen there were significant increases in body curls, face wiping, limb movements, and mouth movements. Quiet behavior, which covaries with the other behaviors, was decreased in the withdrawal condition.



15-sec interval. The second person, also unaware of the treatment condition, recorded the behavior on the behavioral checklist.

Statistics

A factorial analysis of variance (ANOVA) was conducted for each behavior. The 10-min observation period was divided into 2 different time periods, each consisting of 5 min. We did this to assess if there were any time dependent changes in the withdrawal behaviors. There were none, and data are presented for the entire 10-min session. Behaviors were averaged for pups in each litter receiving the same treatment. Therefore the litter was the unit of measure. Averaging the data per litter reduces variability and increases the power of the ANOVA. Naltrexone or saline doses were injected within a single litter, and the two treatments treated as a within-subjects variable. The different chronic treatment groups were a between-subjects variable.

RESULTS

The results demonstrate increased fetal activity precipitated by naltrexone in morphine-exposed animals. No significant time effects were observed. Face wiping occurred in all morphine-exposed animals treated with naltrexone but never occurred in any other treatment group. Therefore, no analysis was performed for this behavior. The following other behaviors showed significant interaction effects between acute naltrexone treatment and chronic treatment (Fig. 1). The interaction is of interest because withdrawal is defined as occurring only in fetuses given chronic morphine and acute naltrexone: Body curls, ($F(1, 7) = 23.51$, $p < 0.001$); forelimb movements, ($F(1, 7) = 25.10$, $p < 0.001$); hindlimb movements, ($F(1, 7) = 11.57$, $p < 0.01$); mouth movements, ($F(1, 7) = 13.94$, $p < 0.01$); and quiet, ($F(1, 7) = 30.08$, $p < 0.001$). Behaviors demonstrating no significant effects were body twitch, head movements, and stretch (Fig. 2).

DISCUSSION

The presence of a withdrawal syndrome in the fetal animal has been previously hypothesized (13,15,16), and motor activity increases in naloxone-treated fetuses of dams exposed chronically to morphine (12). The present study indicates that naltrexone-injected GD 20 fetuses that had been passively exposed to morphine (through the placental transport of the drug) show a specific alteration in behavior patterns and provides a detailed behavioral profile that defines the prenatal opiate abstinence syndrome. We were able to identify the same basic patterns of fetal movement as previously reported by Smotherman and Robinson (21) in their study of spontaneous behavior in the fetal rat. The results of the present study show that behaviors that spontaneously occur in the fetus, such as body curls and mouth and limb movements, were greatly increased in naltrexone injected fetuses chronically exposed to morphine as compared with control animals. Other spontaneous behaviors such as body twitch, head movements, and stretching did not increase significantly in

FIG. 2. Fetal behaviors that showed no signficant interaction effect. The details are as in Fig. 1. These data are the mean number of occurrences ± one SEM of fetal behaviors. Lateral head moves, stretches, and body twitch were not increased in withdrawal.

experimental animals. Face wiping was unique in that it only occurred in morphine-exposed animals treated with naltrexone. This behavior involved a synchronous movement of the two forelegs along the side of the head and closely resembled grooming patterns observed in adolescent and adult rats during withdrawal (11). Smotherman and Robinson (22) characterize face wiping as a pattern of motor behavior rarely seen in the absence of aversive stimulation such as a tactile probe or chemosensory stimulation. Smotherman and Robinson's (22) findings taken together with the present results suggest that face wiping may be a motor response evoked by aversive stimuli.

Several animal models have been used to examine the effects of opiate withdrawal in utero. Lichtblau and Sparber (15) found that litters from dams put through withdrawal daily with naloxone injections from day 14 of gestation through term showed an increased rate of stillbirths, decreased pup weight and size, and weight loss 24 hours after birth as compared with morphine-dependent saline treated rats. When morphine-dependent fetal chicks were injected with naloxone, the motility of the opiate-exposed fetuses was significantly increased and the hatchability significantly reduced as compared with control fetuses (14). Following an injection of naloxone into fetuses from morphine-exposed ewes, fetal arterial blood pressure and defecation increased, and fetal heart rate decreased (6). Pups exposed to methadone prenatally, and thus exposed prenatally to the opiate through the placenta and postnatally through milk, show precipitated withdrawal at 7 days of age (3). The effects of morphine on spontaneous activity in the rat fetus have also been observed. Fetal movements begin on the 15th day of gestation. Kirby (13) injected the dam with morphine on gestation days 7 through 17. Spontaneous fetal activity from days 15 to 21 was decreased at all ages and this decrease was reversed by naloxone.

Quantification of these specific behavioral changes associated with the fetal abstinence syndrome provides some insight into how the effects of opiates and opiate withdrawal on fetal behavior may contribute to the adverse effects observed in offspring of drug-dependent women. For example, past research has emphasized the placental transfer of opiates as the primary mechanism involved in the deleterious effects associated with maternal opiate dependence. Although this mechanism accounts for the direct effects of placental transport (18), it ignores the complexity of the fetal environment, which is constantly interacting with the fetus. Motor activity in the fetus is necessary to promote normal behavioral as well as morphological development (23). It has been previously suggested that these behaviors are not just trivial aspects of the existence of the fetus but rather they are ontogenetic adaptations designed to promote the survival and growth of the fetus (17,23). Therefore anything that influences the behavior of the fetus may be a potential threat to normal development. For example, periodic episodes of heroin withdrawal during pregnancy restrict fetal growth by reducing uterine or placental bloodflow (16). Episodes of maternal withdrawal will increase the activity of the fetus, thereby increasing the metabolic rate and oxygen consumption of the fetus. As a result, the fetus may experience hypoxia or even death (8). Thus the effects caused by opiate withdrawal may be a consequence of both direct and indirect effects of abstinence.

The results of this study indicate that the rat fetus does experience withdrawal from opiates in utero suggesting the possibility that abstinence may be in part responsible for some of the adverse effects of opiates observed in animals as well as humans. Furthermore, the description of behaviors associated with opiate withdrawal in the fetus provides a developmental model to study further the anatomical and physiologic substrates that may mediate this syndrome.

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REFERENCES

1. Advokat, C.: Environmental modulation of analgesic tolerance induced by morphine pellets. Pharmacol. Biochem. Behav. 14:139–142; 1981.
2. Barr, G.A., Goodwin, G.A.: Precipitated morphine withdrawal induces a conditioned aversion in the preweaning rat. Pharmacol. Biochem. Behav. 57:779–783; 1997.
3. Barr, G.A.; Zmitrovich, A.; Hamowy, A.S.; Liu, P. Y.; Wang, S.; Hutchings, D.E.: Neonatal withdrawal following pre- and postnatal exposure to methadone in the rat. Pharmacol. Biochem. Behav. 60:97–104; 1998.
4. Besunder, J.B., Blumer, J.L.: Neonatal drug withdrawal syndromes. In: Koren, G., ed. Maternal-fetal toxicology. New York: Marcel Dekker, Inc.; 1990: 161–177.
5. Cicero, T.J., Meyer, E.R.: Morphine pellet implantation in rats: quantitative assessment of tolerance and dependence. J. Pharmacol. Exp. Ther. 184:404–408; 1973.
6. Cohen, M.S.; Rudolph, A.M.; Melmon, K.L.: Antagonism of morphine by naloxone in pregnant ewes and fetal lambs. Dev. Pharmacol. Ther. 1:58–69; 1980.
7. Fanselow, M.S., Cramer, C.P.: The ontogeny of opiate tolerance and withdrawal in infant rats. Pharmacol. Biochem. Behav. 31:431–438; 1988.
8. Finnegan, L. P.: Clinical effects of pharmacologic agents on pregnancy, the fetus and neonate. Ann. NY Acad. Sci. 281:74–89; 1976.
9. Hans, S.L.; Marcus, J.; Jeemy, R.F., Auerbach, J.G.: Neurobehavioral development of children exposed inutero to opoioid drugs. In: Yanai, J., ed. Neurobehavioral teratology. New York: Elsevier; 1984.
10. Hutchings, D.E.: Issues of risk assessment: lessions from the use and abuse of drugs during pregnancy. Neurotoxicol. Teratol. 12:183–189; 1990.
11. Jones, K.L., Barr, G.A.: Ontogeny of morphine withdrawal in the rat. Behav. Neurosci. 109:1189–1198; 1995.
12. Kirby, M.L.: Effects of morphine and naloxone on spontaneous activity of fetal rats. Exp. Neurol. 73:430–439; 1981.
13. Kirby, M.L.: Effects of morphine on spontaneous activity of 18-day rat fetus. Dev. Neurosci. 2:238–244; 1979.
14. Kuwahara, M.D., Sparber, S. B.: Prenatal withdrawal from opiates interferes with hatching of otherwise viable chick fetuses. Science 212:945–947; 1981.
15. Lichtblau, L., Sparber, S.B.: Opiate withdrawal in utero increases neonatal morbidity in the rat. Science 212:943–945; 1981.
16. Naeye, R.L.: Malnutrition, a probable cause of fetal growth retardation. Arch. Pathol. 79:284–291; 1965.
17. Oppenheim, R.W.: The neuroembryological study of behavior: progress, problems, perspectives. Curr. Top. Dev. Biol. 17:257–309; 1982.
18. Ricalde, A.A., Hammer, R.P. Jr.: Perinatal opiate treatment delays growth of cortical dendrites. Neurosci. Lett. 115:137–143; 1990.
19. Smith, A.A.; Hui, F.W., Crawford, M.J.: Inhibiiton of growth in

youngmice treated with d,l-methadone. Eur. J. Pharmacol. 43:307–314; 1973.
20. Smotherman, W.P.; Robinson, S.R.: Accessibility of the rat fetus for psychobiological investigation. In: Shair, H.N.; Barr, G.A.; Hofer, M.A., eds. Developmental psychobiology: New methods and changing concepts. New York: Oxford University Press; 1991:148–166.
21. Smotherman, W.P., Robinson, S.R.: Behavior of rat fetuses following chemical or tactile stimulation. Behav. Neurosci. 102:24–34; 1988.
22. Smotherman, W. P., Robinson, S.R.: Kappa opioid mediation of fetal responses to milk. Behav. Neurosci. 106:396–407; 1992.
23. Smotherman, W.P., Robinson, S.R.: Prenatal influences on development: behavior is not a trivial aspect of fetal life. J. Dev. Behav. Pediatr. 8:171–176; 1987.
24. Thornton, S.R., Smith, F.L.: Characterization of neonatal rat fentanyl tolerance and dependence. J. Pharmacol. Exp. Ther. 281:514–521; 1997.
25. Thornton, S.R.; Wang, A.F.; Smith, F.L.: Characterization of neonatal rat morphine tolerance and dependence. Eur. J. Pharmacol. 340:161–167; 1997.
26. Windh, R.T.; Little, P.J., Kuhn, C.M.: The ontogeny of mu opiate tolerance and dependence in the rat: antinociceptive and biochemical studies. J. Pharmacol. Exper. Ther. 273:1361–1374; 1995.
27. Yoburn, B.C.; Chen, J.; Huang, T., Inturrisi, C.E.: Pharmacokinetics and pharmacodynamics of subcutaneous morphine pellets in the rat. J. Pharmacol. Exp. Ther. 235:282–286; 1985.