Pharmacology Biochemistry & Behavior, Vol. 39, pp. 389–394. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Opioid-Dependent Behaviors in Infant Rats: Effects of Prenatal Exposure to Ethanol

PRISCILLA KEHOE*[1] AND WILLIAM SHOEMAKER†

**Trinity College, Hartford, CT*
†University of Connecticut Health Center, Farmington, CT

Received 12 September 1990

KEHOE, P. AND W. SHOEMAKER. *Opioid-dependent behaviors in infant rats: Effects of prenatal exposure to ethanol.* PHARMACOL BIOCHEM BEHAV **39**(2) 389–394, 1991.—Pregnant rats were given diets containing either 5% ethanol, an isocaloric (pair-fed) diet, or casein pellets. Offspring were tested at postnatal day 10 for isolation-induced ultrasonic vocalizations and subsequent stress-induced analgesia. Rats prenatally exposed to ethanol vocalized significantly less in the five minutes during isolation. The opiate, morphine, caused a greater suppression of vocalizations in alcohol-exposed pups compared to controls, while the increased calling normally seen with the opiate antagonist, naltrexone, was attenuated. In a test in which the pup withdraws a paw from a hot plate (48°C), prenatal alcohol offspring demonstrated baseline latencies (no isolation) similar to controls but had greatly attenuated responses in their isolation-induced analgesia. Since both vocalization and analgesia responses have been determined to be modulated by the endogenous opioid system, the aberrant responses of the prenatal-ethanol-exposed offspring can be interpreted as failures to respond by opioid release/secretion to appropriate stimuli.

Sprague-Dawley rats Prenatal alcohol Opioid mediation Ultrasonic vocalizations Neonatal analgesia Fetal Alcohol Syndrome (FAS)

PRENATAL alcohol exposure results in a constellation of behavioral and anatomical alterations in affected offspring termed the Fetal Alcohol Syndrome (FAS) (25,37). Human infants of chronic alcoholics demonstrate a variety of developmental difficulties (22,30). Martin, Martin, Streissguth and Lund (16) studied a large group of human FAS offspring and described their suckling behavior as being delayed and displaying a weak suck. Long-term studies of FAS children show that these offspring have difficulty in learning from experience, adjusting their behavior to specific situations and do not respond "normally" to punishment, even as young adults (29). Additionally, in the Ainsworth "strange situation" procedure (1), human infants exposed to moderate and high levels of alcohol prenatally compared to those not exposed show greater disorganized and insecure attachment behavior (21). Furthermore, Chen, Driscoll and Riley (6) have described altered suckling behaviors in rat pups prenatally exposed to ethanol. The exposed pups displayed longer latencies to attach to the nipple, as well as failure to nipple shift (24). Studying the effects of gestational exposure to alcohol on infant learning and emotional development is important, since the early relationship with the caretaker has significant and long-term effects on development.

A number of studies have been carried out that report neurotransmitter alterations in the central nervous system of experimental animals exposed to ethanol prenatally (7). Shoemaker, Baetge, Azad, Sapin and Bloom (26) reported that the endogenous opioid peptide, β-endorphin, is increased in the midbrain and hindbrain regions of newborn FAS rats. In addition, Nelson, Taylor, Lewis, Branch and Liebeskind (21) demonstrated that adult rats prenatally exposed to ethanol demonstrate a greater response than controls to the opiate, morphine, on analgesia tests. Moreover, they reported that FAS offspring as adults display an enhanced analgesic response to an opioid-mediated stressor, footshock (18,19). In another study, prenatal alcohol-exposed males displayed the highest baseline level of analgesia, i.e., prior to stress exposure (2).

Recent research has described the development of neonatal rat behaviors that are dependent on endogenous opioid systems for their normal expression (9, 11–15). These opioid-mediated behavioral systems can be activated by stressful situations such as isolation from the mother. In the case of the neonatal rat, isolation from the mother produces a distress response including ultrasonic vocalizations. It has been demonstrated that the endogenous opioid systems are responsible for the quieting that occurs after 5 minutes of isolation distress (14). Moreover, the opioid systems are important in neonatal pain sensitivity and can be modulated with exogenous opiates and various stressors (12, 14, 27). While opioid systems are thought to play a major role in calling behavior and analgesia, other neurotransmitter systems are likely involved in these complex processes, (4, 5, 10, 15, 17, 27) some or all of which may be affected by prenatal alcohol exposure.

Because infants prenatally exposed to ethanol are deficient in stress adaptation behaviors, the present study assessed gestationally exposed neonatal rats for opioid-mediated behaviors that included isolation-induced ultrasonic vocalizations and subsequent morphine- and stress-induced analgesia. Morphine, an opiate that mimics the effects of the endogenous peptides, has been shown

[1]Requests for reprints should be addressed to Dr. P. Kehoe, Department of Psychology, Trinity College, Hartford, CT 06106.

to suppress isolation-induced vocalizations and reduce pain sensitivity (12–14). In addition, naltrexone, an opioid receptor antagonist, increases ultrasonic vocalizations of the isolate and decreases pain sensitivity (12–15). These drugs are used to define the status of the endogenous opioid systems following the various prenatal treatments.

METHOD

Subjects

Time-mated Sprague-Dawley female rats obtained from Zivic-Miller Labs, Pittsburgh, PA, and their offspring were the experimental subjects. The pregnant females were fed one of three experimental diets from gestational day (gd) 7 until gd 20. The females were housed singly in plastic cages with wood shavings used as bedding. Animal rooms were maintained at 22°C with a 12-hour dark/light cycle (5 p.m./5 a.m.).

Within 2–4 hours of birth, all pups were fostered to lactating females that were 1 to 2 days postpartum and were maintained on control chow pellet diets ad lib. Litters were culled to 10 pups per foster mother, with an effort to maintain an equal number of males and females per litter. When more than 2 pups died in a litter during the first 10 days postnatal, pups from similar treatment mothers were combined to maintain litter size from 8–10. One hundred and seventy 10-day-old pups representing at least 7 litters for each condition were used. Day of birth was considered Day 0. No more than one pup per litter was studied for any one treatment condition.

Pregnancy and Postpartum Parameters

A number of parameters were measured in the dam during gestation and parturition: blood alcohol levels, time of parturition and litter size. In regard to the offspring, we recorded appearance at birth, birth weight and mortality or morbidity information. Observations of maternal behaviors were made several times a day during the postnatal period to determine the quality of caretaking the pups in each treatment group received.

Blood Alcohol Determination

All mothers, whether they received alcohol or not, were bled once during gestation at 9 p.m. on gd 19. Samples of mixed arterial and venous blood were obtained from unanesthetized dams by nicking the tail with a razor blade. Blood alcohol levels were assayed by a micromodification of the alcohol dehydrogenase method using the Sigma Alcohol Kit (Sigma Diagnostics, St. Louis, MO).

Diet

The alcohol-containing liquid diet (BSA) contains 5% ethanol and has the following composition as a percent of total kilocalories: protein 17%, carbohydrate 12%, fat 36%, and ethanol 36%. This diet was chosen from a comparison of several liquid diets based on maintaining the highest blood alcohol levels without producing abortions or failure to deliver (31). The pair-fed diet (BSP) contained no alcohol but was isocaloric to the BSA with the kilocalories made up by maltose-dextrin. The ingredients for all liquid diets were purchased from Bio-Serv, Inc., Frenchtown, NJ (38). A control chow pellet diet (Control) in which casein is used as the protein source was obtained from Teklad Mills, Madison, WI (Teklad #170480). The pregnant females self-administered the alcohol by ingesting a liquid diet that contains 5% ethanol (38). The alcohol diet group was then paired with a weight-matched group that received the liquid diet without ethanol (pair-fed group); a chow pellet control group was also included in the design (31).

Drug Treatment on Postnatal Day 10

All pups were given one of three solutions injected in a volume of 0.05 ml intraperitoneally. One group received an isotonic saline injection, 0.05 ml, a second group received an injection of naltrexone, 0.5 mg/kg, and the third group received an injection of morphine, 0.5 mg/kg. The doses administered were chosen because previous studies have shown that these doses were optimal for reliable behavioral changes at this age (12–15). Following the injection, each pup was returned to the dam.

Isolation Treatment and Vocalization Measurements

Since rat pups do not vocalize in the nest, even with naltrexone treatment (15), one-half of each drug treatment group was subjected to a 5-minute isolation period in an environmental chamber maintained at 32°C. Fifteen minutes after the drug injection the pup was placed in a styrofoam cup that contained 20 cc of clean bedding. During this time, the pup's ultrasonic vocalizations, made audible through a QMC Instruments Bat Detector, were counted and recorded. Rats were then tested for response to nociception on a hot plate as described below. All tests were done by experimenters who were blind to the pup's prenatal diet or postnatal drug treatment.

Nociception Testing

All pups, isolated or not, were tested for responsivity to heat. Those not isolated were injected with a drug, returned to the nest for 15 min and then taken directly from the nest for analgesia testing. Each rat was gently lifted and positioned such that its left front paw rested first on a table top for 10 seconds and then contacted a heated surface (48°C) (Analgesia Meter, Columbus International Instruments Corp., Columbus, OH). A foot pedal was depressed by the experimenter when the pup made paw contact with the heated surface and lifted when the pup's paw was withdrawn. A maximum time of 40 seconds was allotted to avoid any tissue damage to the pup's paw.

Statistical Analyses

Analysis of variance was performed on the number of vocalizations and paw removal latency of pups in each condition. Statistically significant effects were further analyzed by the Newman-Keuls test for post hoc pairwise comparisons (39).

RESULTS

Maternal Parameters

As has been reported (31, 34, 38), feeding an alcohol-containing diet to pregnant rats results in a certain number of stillborn offspring and a high percentage of neonatal deaths between birth and postnatal Day 7 (Table 1). This neonatal mortality occurs despite very early culling of the litters and placement of the pups with normal-diet foster mothers. The causes of this mortality remain obscure. Nevertheless, the surviving pups display little morbidity as assessed by locomotion, sensory respon-

TABLE 1

	BSA	BSP	PC
No. of Litters	18	8	8
No. of Live Births	214	92	109
No. Stillborn	3	0	0
% Mortality Birth-PN7	27%	0	0
Parturition Delay	20 hr	0	0
Avg. Body Weight at Birth	5.56 g	6.20 g	6.72 g
Blood Ethanol of Dam	225 mg/dl		

siveness, growth rate and nipple attachment behavior (unpublished observations).

Blood Alcohol Levels of the Dams

The blood alcohol levels of those mothers receiving alcohol-containing diets were measured on gestation day 19 at 9 p.m., a point of maximum level after onset of food ingestion. Mothers on pair-fed or chow pellet control diets had no measurable blood alcohol. In this study, the BSA mothers had a mean blood alcohol level of 225 mg/dl, a moderate but not sedative level of ethanol (Table 1). This level is consistent with other reported levels (31,38).

Birthweight

The deficit in weight found in the BSA animals at birth (Table 1) continues past weaning (31,38).

Isolation Vocalizations

The number of vocalizations emitted by the isolated pup was significantly affected by the interaction of the prenatal diet and drug administration, $F(4,76)=3.6$, $p<0.0001$ (Fig. 1). Compared to chow pellet (Control) or pair-fed dams (BSP), pups exposed to alcohol prenatally (BSA) and administered saline on Day 10 vocalized significantly less, i.e., 140, 125 and 49 vocalizations, respectively, $F(2,28)=4.7$, $p<0.01$ (Newman-Keuls, critical difference = 70, $p<0.05$). Similarly, the morphine treatment produced a much greater reduction in vocalizations in the alcohol-exposed pups (11) than either the control (54) or pair-fed pups (44), $F(2,25)=6.6$, $p<0.001$ (Newman-Keuls critical difference = 28, $p<0.05$). Additionally, when comparing morphine with saline administration, the BSA condition is more affected, demonstrating a greater percentage reduction of calling due to morphine than the other prenatal conditions (BSA = 78%, BSP = 65% and Control = 61%).

The naltrexone treatment caused a profile that contrasted to that of saline and morphine administration in that the alcohol-treated (148) and pair-fed offspring (181) vocalized significantly less than the chow pellet controls (360), $F(2,23)=4.9$, $p<0.01$ (Newman-Keuls critical difference = 148, $p<0.05$).

These data demonstrate that prenatal alcohol exposure results in a markedly reduced vocalization response to isolation in the 10-day-old pup. The results of the alcohol prenatal treatment group would appear to be a combination of the ethanol exposure and the concomitant undernutrition that accompanies it. The pair-fed group displayed some deficiency in calling behavior, at least that provoked by blocking opioid receptors with naltrexone.

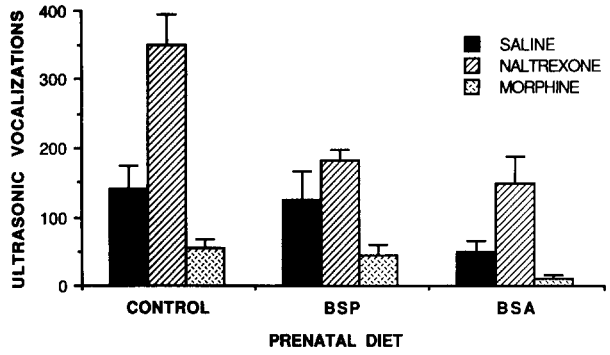

FIG. 1. Day 10 isolation vocalizations. Mean number of ultrasonic vocalizations for each prenatal treatment group (chow pellet control, pair-fed BSP, and alcohol diet BSA) monitored on Day 10 during 5 minutes of isolation 15 minutes after receiving an IP injection of saline, naltrexone (0.5 mg/kg) or morphine (0.5 mg/kg).

Analgesia

The latency for the pup to withdraw its paw from the heated surface was significantly affected by the interaction of drug administration and diet, $F(4,152)=5.5$, $p<0.0001$, diet and isolation, $F(2,152)=3.0$, $p<0.05$, and drug administration and isolation, $F(2,152)=4.9$, $p<0.01$ (Fig. 2).

There were no statistical differences between the prenatal diet groups in terms of latency to remove a paw from heat when the pups were given saline (Fig. 2) or naltrexone (data not shown) in the nonisolation condition.

Compared to the chow pellet control and pair-fed groups, the isolation saline condition resulted in a significant decrease in latency in the alcohol-exposed pups 17.7, 15.5, and 11.1 seconds, respectively, $F(2,28)=10.5$, $p<0.001$ (Newman-Keuls critical difference = 4.9, $p<0.05$) (Fig. 2). Compared to nonisolated saline rat pups, the isolated saline subjects demonstrated an analgesia to heat after isolation stress, $F(2,54)=8.5$, $p<0.0001$

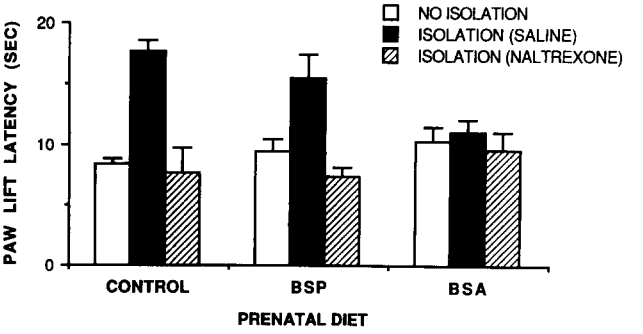

FIG. 2. Day 10 test for analgesia. Mean paw removal latency (s) from a 48°C hot plate for each prenatal treatment group (chow pellet control, pair-fed BSP, and alcohol diet BSA) tested either directly from the nest (nonisolation) or at the end of the 5-minute isolation period, prior to which an IP injection of saline or naltrexone 0.5 mg/kg had been administered.

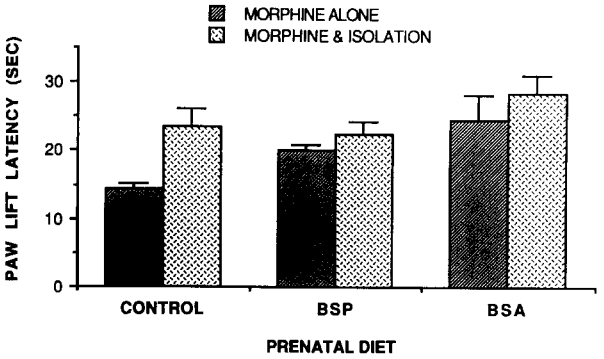

FIG. 3. Analgesia response to morphine and isolation. Mean paw removal latency (s) from a 48°C hot plate for each prenatal treatment group (chow pellet control, pair-fed BSP, and alcohol diet BSA) tested after an injection of IP morphine (0.5 mg/kg) either directly from the nest or following a 5-minute isolation period.

(Newman-Keuls critical difference = 3.6, $p<0.05$), a response not seen in pups prenatally exposed to alcohol.

Pretreatment with naltrexone prior to isolation blocked the isolation-induced change in pain sensitivity seen in saline-administered control and BSP groups, $F(2,53)=5.3$, $p<0.01$ (Newman-Keuls critical difference = 3.3, $p<0.05$), causing latencies similar to those found in the nonisolated siblings (Fig. 2). While alcohol-treated pups were similar to both chow pellet control and pair-fed groups in heat withdrawal latency without isolation or after isolation with naltrexone administration, they did not demonstrate the isolation-induced change in pain sensitivity. Because this change in pain threshold is naltrexone reversible, it appears that the alcohol-exposed pups may be deficient in the opioid mediation of this analgesic response to isolation.

As seen in Fig. 3, a somewhat different profile is seen with morphine administration alone, in which the BSA and BSP were significantly slower at paw removal (24.6 and 20.0 seconds, respectively) than the chow-fed controls (14.4 seconds), $F(2,21)=5.0$, $p<0.01$ (Newman-Keuls critical difference = 5.2, $p<0.05$). While this seems to be in concert with the results found by Nelson et al. (20), who tested prenatally exposed pups as adults and found a potentiated response to morphine, the response of the pair-fed offspring may point to a role of prenatal stress or nutritional factors in this behavior.

Another instance in which prenatally exposed pups would seem to respond to the isolation stress by minimal endogenous opioid secretion is seen with morphine administration combined with isolation (Fig. 3). Control pups given morphine and then isolated demonstrated latencies somewhat longer than those not isolated, $F(1,46)=5.5$, $p<0.01$ (Newman-Keuls critical difference = 6.8, $p<0.05$). This effect was not evident in the pair-fed and BSA groups, since they showed longer latencies than chow pellet controls with morphine alone and then only a small increment when isolation was added to the drug treatment.

DISCUSSION

In the present study, we describe deficits in two additional neonatal behaviors seen in offspring of alcohol-ingesting mothers. Tested at 10 days postnatal, infant rats prenatally exposed to ethanol are deficient in calling behavior when separated from the nest and deficient in their stress-related pain sensitivity mechanisms.

To what extent are these results due to prenatal ethanol per se rather than to the experimental procedures (i.e., liquid diet, reduced food intake, daily handling)? The inclusion of the pair-fed group (BSP) that received a restricted amount of nonalcoholic liquid diet is an attempt to provide information on this point. Although the calling behavior during isolation is reduced in the prenatal ethanol group and not in the pair-fed offspring, the response to naltrexone administration (Fig. 1) indicates that the treatments imposed in the pair-feeding procedure produce abnormalities in some parameters. A number of studies have described abnormal stress responsiveness and brain opioid changes following neonatal undernutrition (23, 32, 33). Thus the small amount of undernutrition and probable stress imposed by the pair-feeding paradigm (Table 1) may produce abnormalities in these parameters independent of ethanol's effects. In addition, all of these treatment groups underwent a certain amount of stress during shipment and bleeding procedures. While these stresses could contribute to treatment outcome, vocalization and analgesia behaviors of control offspring in this study are comparable to levels of these behaviors of offspring from laboratory-bred, nonbled mothers.

In general, alcohol-exposed pups are smaller than control pups at birth and on postnatal Day 10. We were concerned that the decrease in vocalizations was related to the lower body weight. When correlating the number of calls with body weight within each treatment group, however, no significant correlations were found. Additionally, there is significant overlap in body weights in that many BSA pups are equal in size to control pups and yet vocalize considerably less.

Comparisons among chow pellet control, pair-fed, and ethanol groups are one way to dissociate the effects of ethanol and stress of undernutrition. A closer examination of the response profiles of the pair-fed offspring compared to the chow pellet control group suggests that the differences appear chiefly in response to the pharmacological agents employed in the design (e.g., naltrexone response in Fig. 1, morphine response in Fig. 3). This implies that what may be altered in the pair-fed offspring is related to the opiate receptors involved in mediating these drug responses.

In contrast to the pair-fed group, the abnormalities seen in the alcohol-exposed offspring (BSA) appear to be due to failure to respond to the isolation stress by releasing or secreting endogenous opioids. For instance, while there was no difference in baseline analgesia levels in the three groups (no isolation condition in Fig. 2), the BSA offspring fail to demonstrate the increased latency reliably produced by the 5-minute isolation. That the increased latency to paw lift following isolation stress is opioid mediated can be seen by the nearly complete block produced by the naltrexone injection (Fig. 2). Another example of the failure of the BSA group to augment its baseline analgesia following the stress is seen in Fig. 3, where the added latency to morphine is significant in the control group but not in the other treatment groups. As discussed above, baseline antinociception may be due to the concerted action of several nervous system pathways utilizing many neurotransmitters. The augmentation of the paw lift latency, however, appears to be due chiefly to endogenous opioid mediation, since it is completely blocked by the opiate antagonist.

A possible interpretation of these results is that the enhanced latencies are due to a stress-induced release of the endogenous opioid from nerve terminals at selected synaptic sites within the brain. A recent report has measured β-endorphin levels in several brain regions following a footshock stress in adults (3). In that study, rats prenatally exposed to ethanol failed to respond to stress by releasing the β-endorphin in certain brain regions, as did the controls. It is not currently known whether the endogenous opioid involved in the isolation-induced analgesia at postnatal day 10 is, in fact, β-endorphin rather than, or in addition

to, enkephalin or dynorphin peptides.

In regards to the involvement of the opioid receptors, the results in Fig. 3 suggest that the prenatal ethanol treatment renders the offspring hyperresponsive to morphine, as has been reported by Nelson et al. (20) in adults. Thus a possible explanation of at least some of these observations is increased sensitivity at one or more of the opiate receptors.

Stress responsiveness in ethanol-exposed offspring may, in fact, be dependent on the type of stressor to which they are subjected (2, 19, 35, 36). In the present study, separation from the dam and siblings for 5 minutes is a stressor that pups respond to immediately, within 3 minutes, with opioid-mediated behaviors (14). It is possible that the alcohol-exposed pups may be deficient in responding to such a stressor by not perceiving individual isolation as do the control pups. The pup does, however, vocalize when isolated, apparently recognizing the isolation state, but the rate of calling is low. The initiation of vocalizing may be due to the mediation of the norepinephrine system (15), a neurotransmitter reported to be deficient in the FAS offspring (7,26). While these vocalization abnormalities may be derived from CNS exposure to alcohol, they are likely confounded by postnatal mother-infant interactions that might be qualitatively or quantitatively inferior due to the pup's behavioral deficiencies in the nest (28).

In conclusion, we believe the studies reported here further implicate one or more of the endogenous opioid systems as being defective following prenatal ethanol exposure. As details of the functions of these systems emerge, and the precise nature of the defect is characterized, the role of opioid peptides in pain sensitivity, stress responsiveness, and social behavior may be learned. This knowledge could have implications for the clinical management of children and adult offspring prenatally exposed to ethanol.

ACKNOWLEDGEMENTS

This work was supported by grants 2P50-AA03510 and 5R01-AA06927 of the National Institute of Alcoholism and Alcohol Abuse. We would like to thank Dr. Eva Vavrousek-Jakuba, Ross Baker, Carolyn Boylan and Chantal Bade for their help and suggestions during the course of this study.

REFERENCES

1. Ainsworth, M. D. S.; Blehar, M. C.; Waters, E.; Wall, S. Patterns of attachment. Hillsdale, NJ: Lawrence Erlbaum Associates; 1978.
2. Baker, R. A.; Shoemaker, W. J. Effect of prenatal ethanol on adult opioid mediated analgesia. Soc. Neurosci. Abstr. 13:919; 1987.
3. Baker, R. A.; Shoemaker, W. J. The effect of acute stress on levels of immunoreactive β-endorphin in discrete brain regions in rats. Soc. Neurosci. Abstr. 15:846; 1989.
4. Basbaum, A. I.; Fields, H. L. Endogenous pain control systems: Brainstem spinal pathways and endorphin circuitry. Annu. Rev. Neurosci. 7:309–338; 1984.
5. Carden, S. E.; Hofer, M. A. Independence of benzodiazepine and opiate action in the suppression of isolation distress in rat pups. Behav. Neurosci. 104:160–166; 1990.
6. Chen, J. S.; Driscoll, C. D.; Riley, E. P. Ontogeny of suckling behavior in rats exposed to alcohol prenatally. Teratology 26:145–153; 1982.
7. Druse, M. J. Effects of perinatal alcohol exposure on neurotransmitters, membranes, and proteins. In: West, J. R., ed. Alcohol and brain development. New York: Oxford University Press; 1986:343–372.
8. Hunt, E. H.; Smotherman, W. P.; Wiener, S. G.; Levine, S. Nutritional variables and their effect on the development of ultrasonic vocalizations in rat pups. Physiol. Behav. 17:1037–1039; 1976.
9. Kehoe, P. Opioids, behavior, and learning in mammalian development. In: Blass, E. M., ed. Developmental psychobiology and behavioral ecology. 9th ed. New York: Plenum Press; 1988:309–340.
10. Kehoe, P. The neuropharmacology of neonatal rat's separation vocalizations. In: Zinder, O.; Bresnitz, S., eds. Molecular biology of stress: UCLA symposium on molecular and cellular biology. vol. 97. New York, NY: Alan R. Liss, Inc.; 1989:307–317.
11. Kehoe, P.; Blass, E. M. Behaviorally functional opioid systems in infant rats: I. Evidence for olfactory and gustatory classical conditioning. Behav. Neurosci. 3:359–367; 1986.
12. Kehoe, P.; Blass, E. M. Behaviorally functional opioid systems in infant rats: II. Evidence for pharmacological, physiological, and psychological mediation of pain and stress. Behav. Neurosci. 5:624–630; 1986.
13. Kehoe, P.; Blass, E. M. Central nervous system mediation of positive and negative reinforcement in neonatal albino rats. Dev. Brain Res. 27:69–75; 1986.
14. Kehoe, P.; Blass, E. M. Opioid-mediation of separation distress in 10-day-old rats: Reversal of stress with maternal stimuli. Dev. Psychobiol. 19:385–398; 1986.
15. Kehoe, P.; Harris, J. C. Ontogeny of noradrenergic effects on ultrasonic vocalizations in rat pups. Behav. Neurosci. 5:1099–1107; 1989.
16. Martin, D. C.; Martin, J. C.; Streissguth, A. P.; Lund, C. A. Sucking frequency and amplitude in newborns as a function of maternal drinking and smoking. In: Gallanter, M., ed. Currents in alcoholism. vol. 5. New York: Grune and Stratton; 1979:359–366.
17. Mayer, D. J.; Price, D. D. Central nervous system mechanisms of analgesia. Pain 2:379–404; 1976.
18. Nelson, L. R.; Lewis, J. W.; Liebeskind, J. C.; Branch, B. J.; Taylor, A. N. Fetal exposure to ethanol potentiates opioid stress analgesia in adult rats. Soc. Neurosci. Abstr. 8:596; 1982.
19. Nelson, L. R.; Taylor, A. N.; Lewis, J. W.; Branch, B. J.; Liebeskind, J. C. Opioid but not nonopioid stress-induced analgesia is enhanced following prenatal exposure to ethanol. Psychopharmacology (Berlin) 85:92–96; 1985.
20. Nelson, L. R.; Taylor, A. N.; Lewis, J. W.; Branch, B. J.; Liebeskind, J. C. Morphine analgesia potentiated in adult rats prenatally exposed to ethanol. Brain Res. 372:234–240; 1986.
21. O'Connor, M. J.; Sigman, M.; Brill, N. Disorganization of attachment in relation to maternal alcohol consumption. J. Consult. Clin. Psychol. 55:831–836; 1987.
22. Oullette, E. M.; Rosett, H. L.; Rosman, N. P.; Weiner, L. Adverse effects of offspring of maternal alcohol abuse during pregnancy. N. Engl. J. Med. 297:528–530; 1977.
23. Perry, M. L. S.; Carrasco, M. A.; Dias, R. D.; Izquierdo, I. β-Endorphin like immunoreactivity of brain, pituitary gland and plasma of rats submitted to postnatal protein malnutrition: Effect of behavioral training. Peptides 5(1):15–20; 1984.
24. Riley, E. P.; Rockwood, G. A. Alterations in suckling behavior in preweanling rats exposed to alcohol prenatally. Nutr. Behav. 1:289–299; 1984.
25. Rosett, H. L.; Weiner, L. Alcohol and the fetus. New York: Oxford University Press; 1984.
26. Shoemaker, W. J.; Baetge, G.; Azad, R.; Sapin, V.; Bloom, F. E. Effects of prenatal alcohol exposure on amine and peptide neurotransmitter system. Monogr. Neural Sci. 9:130–139; 1983.
27. Spear, L. P.; Enters, E. K.; Aswad, M. A.; Louzan, M. Drug and environmentally-induced manipulations of the opiate and serotonergic systems after nociception in neonatal rat pups. Behav. Neural Biol. 44:1–20; 1985.
28. Stern, J. M.; Johnson, S. K. Ventral somatosensory determinants of nursing behavior in Norway rats. I. Effects of variations in the quality and quantity of pup stimuli. Physiol. Behav. 47:993–1011; 1990.
29. Streissguth, A. P.; Randels, S. Long term effects of fetal alcohol syndrome. In: Robinson, G. C.; Armstrong, R. W., eds. Alcohol and child/family health. Vancouver, BC: University of British Columbia; 1988:135–151.
30. Ulleland, C. N. The offspring of alcoholic mothers. Ann. NY Acad. Sci. 197:167–169; 1972.

31. Vavrousek-Jakuba, E. M.; Baker, R. A.; Shoemaker, W. J. Effects of ethanol on maternal and offspring characteristics: Comparison of three liquid diet formulations fed during gestation. Alcohol.: Clin. Exp. Res.; in press.
32. Vendite, D.; Rocha, J. B. T.; Souza, D. O. Effects of undernutrition during suckling and of training on the hypothalamic β-endorphin of young and adult rats. Peptides 9(4):751–755; 1988.
33. Vendite, D.; Wofchuk, S.; Souza, D. O. Effects of undernutrition during suckling on footshock escape behavior and on related neurochemical parameters in rats. J. Nutr. 115:1418–1424; 1985.
34. Weinberg, J. Effects of ethanol and maternal status on fetal development. Alcohol.: Clin. Exp. Res. 9:73–83; 1985.
35. Weinberg, J. Pituitary-adrenal development following prenatal ethanol exposure. Alcohol.: Clin. Exp. Res. 9:190; 1985.
36. Weinberg, J.; Nelson, L. R.; Taylor, A. N. Hormonal effects of fetal alcohol exposure. In: West, J. R., ed. Alcohol and brain development. New York: Oxford University Press; 1986.
37. West, J. R., ed. Alcohol and brain development. New York: Oxford University Press; 1986.
38. Wiener, S. G.; Shoemaker, W. J.; Koda, L. Y.; Bloom, F. E. Interaction of ethanol and nutrition during gestation: Influence on maternal and offspring development in the rat. J. Pharmacol. Exp. Ther. 216:576–579; 1981.
39. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1971.