ETHANOL-MORPHINE INTERACTIONS : A STUDY OF THE EFFECTS OF PRENATAL EXPOSURE ON THE DEVELOPMENT AND BEHAVIOR OF THE RAT*

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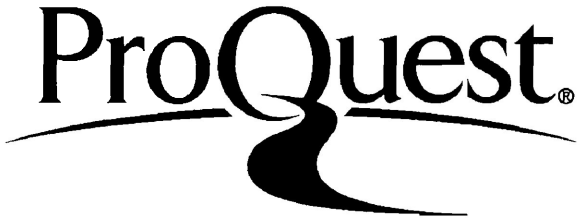

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I dedicate this thesis to my parents Yianni, and Maria.

Abstract

TSOUKATOS, J. YIANNIS (1989). Ethanol-Morphine interactions: a study of the effects of prenatal exposure on the development and behavior of the rat.

The purpose of the study was to investigate the effects of ethanol, morphine and ethanol-morphine on the physical and behavioral development of the rat. Eighteen female and 18 male animals from Satinder's Heterogeneous Stock (SHS) were paired. The dams were then exposed to a regimen of either water, or morphine (0.5 mg/ml), or ethanol (10% v/v), or ethanol (10%v/v)-morphine (0.5mg/ml) (high), or ethanol (5% v/v)-morphine (0.25mg/ml) (low), for 21 days during gestation. The 116 surviving offspring were observed daily until weaning (day 28 postpartum) for physical, reflex, and sexual development. Following weaning the same offspring were tested for preference towards ethanol and morphine, avoidance learning, reactivity, and hot-plate nociception. There were two instances of delayed physical development one due to ethanol, and one due to ethanol-morphine (high) exposure. There were significant developmental delays due to morphine exposure, and delayed female sexual development due to ethanol, and ethanol-morphine (high) exposure. An overall preference for ethanol was observed regardless of prenatal exposure. This tendency was reversed following morphine, or ethanol-morphine (high) exposure. The consumption of either morphine or ethanol on forced and choice days was dependent on the order of presentation. The postnatal

presentation of ethanol and morphine altered food consumption in an order-related pattern. Differences due to prenatal exposure were also observed for the One-Way Avoidance response. It is suggested that the prenatal exposure to ethanol and morphine did not significantly affect learning, while the combination of both agents produced a dose-related hyperresponsivity. The reactivity and the hot-plate testing paradigms were the least sensitive indicators of ethanol-, and/or morphine-induced behavioral effects. The lack of differences is attributed to the limitations of the two testing situations. It is therefore recommended that reactivity testing be extended, and that the hot-plate testing be used in conjunction with postnatal presentation of ethanol or morphine.

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INTRODUCTION AND LITERATURE REVIEW

The issues of prenatal ethanol (etOH) and opiate exposure will be presented as they relate to the postnatal development and behavior of the neonatal rat. The relationship of etOH with opiates will be discussed on the level of the functions of the Endogenous Opiate System (EOS). The theoretical possibilities for the study of etOH and opiate interactions on the development and behavior will be examined.

ETHANOL AS A MODEL TERATOGEN

Fetal Alcohol Syndrome (FAS) and the Diversity of EtOH Action

The use of etOH during pregnancy has been linked with a high risk of offspring being born with Fetal Alcohol Syndrome (FAS). The intensity of the prenatal exposure determines the effect on both the morphology and the behavior of the offspring (Ernhart, Sokol, Martier, Moron, Nadler, Ager, and Wolf, 1987). The importance of proper classification of the FAS symptoms has been recognized by Sterling and Smith (1977). Accordingly, these authors have suggested the distinction between the principal as well as the associated features of the FAS. As principal features they defined the dysfunctions of the Central Nervous System (intellectual, neurologic, and behavioral), the prenatal and postnatal growth deficiencies, and the facial

malformations. As associated features of FAS, were defined the less extensive physical deformities that can be observed in the eyes, ears, mouth, heart, kidneys, skin, bones, and muscles (refer to Tables 1 and 2).

Table 1. Principal Features of the Fetal Alcohol Syndrome Observed in 245 Persons Affected.

Feature	Manifestation
Central-nervous-system dysfunction:	
Intellectual	Mild to moderate mental retardation*
Neurologic	Microcephaly*
	Poor co-ordination, hypotonia†
Behavioral	Irritability in infancy*
	Hyperactivity in childhood†
Growth deficiency:	
Prenatal	<2 SD for length & weight*
Postnatal	<2 SD for length & weight*
	Disproportionately diminished adipose tissue†
Facial characteristics:	
Eyes	Short palpebral fissures*
Nose	Short, upturned†
	Hypoplastic philtrum*
Maxilla	Hypoplastic†
Mouth	Thinned upper vermilion*
	Retrognathia in infancy*
	Micrognathia or relative prognathia in adolescence†

*Feature seen in >80% of patients. †Feature seen in >50% of patients.

Table 2. Associated Features of the Fetal Alcohol Syndro Observed in 245 Persons Affected.

Area	Frequent*	Occasional†
Eyes	Ptosis, strabismus, epicanthal folds	Myopia, clinical microphthalmia, blepharophimosis
Ears	Posterior rotation	Poorly formed concha
Mouth	Prominent lateral palatine ridges	Cleft lip or cleft palate, small teeth with faulty enamel
Cardiac	Murmurs, especially in early childhood, usually atrial septal defect	Ventricular septal defect, great-vessel anomalies, tetralogy of Fallot
Renogenital	Labial hypoplasia	Hypospadias, small rotated kidneys, hydronephrosis
Cutaneous	Hemangiomas	Hirsutism in infancy
Skeletal	Aberrant palmar creases, pectus excavatum	Limited joint movements, especially fingers & elbows, nail hypoplasia, especially 5th, polydactyly, radioulnar synosto pectus carinatum, bifid xiphoid Klippel-Feil anomaly, scoliosis
Muscular		Hernias of diaphragm, umbilicus or groin, diastasis recti

*Reported in between 26 & 50% of patients.
†Reported in between 1 & 25% of patients.

(From Sterling, and Smith, 1978.)

The prenatal exposure to etOH however may produce a continuum of symptoms that are extremely difficult to differentiate among the principal or associated features. To account for these possibilities the two classifications presented by Sterling and Smith have been reorganized into FAS and, Fetal Ethanol Exposure (FEE) (Abel, 1984). More appropriately Zimmerberg and Riley (1986) have argued that FAS is the extreme expression of FEE. The FAS simply constitutes the most severe form of prenatal etOH affliction. Therefore the profile, and severity of each case of FAS or FEE, is determined by individual factors.

Despite the necessity to model the more severe effects of prenatal etOH exposure researchers have also discussed the need to predict any behavioral deficits, whose subtle nature may allow them to go undetected (Norton, 1978). Hutchings (1985), described the continuum of these postnatal symptoms. The observed profiles include mild to severe hyperactivity, distractability, attention, reaction time, and cognitive deficits. One may argue at this point that the study of such deficits is limited to the human species. According to Hutchings (1985) though, similar conditions could be reproduced in animal studies, therefore making the use of animal models possible. The diverse action of ethanol and the subsequent expression of FAS (or FEE) has been greatly facilitated by the use of such models.

The Models and Factors Describing EtOH Teratogenicity

Extensive discussions have been presented on the dual nature of etOH as both a drug and a nutrient. Specifically each gram of etOH produces 7.1 calories during its catabolism (Abel, 1980). The resulting energy has been shown to reduce the apetitte for food and it is considered as the major cause of malnutrition among alcoholics. However the etOH alone, and not the accompanying malnutrition, is responsible for the expression of FAS in humans. Children of undernourished mothers, who did not abuse etOH, did not show FAS-associated symptoms (Sterling and Smith, 1977). This finding has also been confirmed in animal studies (Fernandez, Caul, Hanlein and Vorhees, 1983; Testar, Llobera, and Herrera, 1988).

Robinson in 1977 linked the etOH teratogenicity with the etOH effects on carbohydrate, fat, and protein metabolisms. Some of the neurological effects he assumed were associated with the preventative effects of etOH on serotonin re-uptake from the nerve endings. Other effects were also possible as the ingestion of etOH was accompanied by hypoglycemia, ketosis, and lacticacidaemia. Ever since, additional evidence has accumulated on the effects of prenatal etOH exposure on the postnatal development. Ethanol has been found to alter the physical, the behavioral, and the cognitive development of the offspring.

The Effects of Prenatal EtOH on the Physical and Behavioral Development

The overall physical development of rat pups exposed to etOH prenatally has been found to be delayed. The "episodic" exposure to ethanol during peak brain growth has been shown to be equally disruptive as chronic exposure during a major portion of the brain growth spurt (Burns, Kruckeberg, Kanak, and Stibler, 1986). In addition, body length measures, body weights, and brain or cerebellum weights were reduced (Nathaniel, Nathaniel, Mohamed, Nahybida, and Nathaniel, 1986). Abel and Dintcheff (1978) had reported similar findings. Furthermore they found that pups exposed to low doses of etOH were more likely to exhibit postnatal "catch up" growth than animals exposed to high doses. Research was also conducted in order to correlate the physical aberrations with other developmental deficits due to FEE.

Middaugh, Randall, and Farara (1988) reported that C57 mice (a strain sensitive to the effects of ethanol), exhibited weight reductions near weaning and into adulthood. Although the investigators could not detect differences in the motor activity between the exposed and the non-exposed offspring, the exposed animals were more susceptible to neonatal mortality. However Randall, Becker, and Middaugh (1986) had earlier observed both

hyperactivity and a deficit in acquisition and performance of a shuttle-avoidance task, in the C57 mice. Slightly different administration procedures were employed in these two studies. It is therefore possible that the observed differences were mainly because the two methods resulted in different Blood Ethanol Concentrations, which in turn have been shown to alter the teratogenic risk of etOH (Pierce, and West, 1986).

The teratogenic risk of etOH has also been estimated for the neonatal behaviors that when affected may undermine the normal development of the organism. For instance etOH has been reported to alter the suckling patterns, and delay the development of the surface righting reflex in rat pups regardless of the length, or the onset of the exposure, (Rockwood and Riley, 1986; Viglieccа, Moyano, and Molina, 1986).

Exposure to etOH has been associated with a state of hyperactivity, expressed during the neonatal sleep. Hilakivi (1986) found that perinatally (at 6, 8, 12, and 15 days of age) etOH-exposed offspring had less active sleep and more "wakefulness" than control animals. In addition the quiet states were more often interrupted by waking episodes. The author also reported that the same offspring showed increased voluntary consumption of etOH at two months of age. Taylor, Nelson, Branch, Kokka, and Poland (1984) had also reported that

greater activity of the hypothalamo-pituitary-adrenal (HPA) axis was increased among etOH-exposed offspring. In addition to enhanced analgesia, etOH-exposed rats also exhibited increased etOH consumption, when exposed to stress. Hwang (1986) associated the HPA-related hyperactivity with the increased catecholaminergic synaptogenesis in the cerebral cortex, and the hypothalamus.

EtOH has also been reported to differentially affect the offspring's sexual and related development. Sexual maturation is delayed in the female rat (Esquifino, Sanchis, and Guerri,1986). Furthermore a variety of deficits were observed in the maternal behaviors of etOH-exposed females, while paternal behaviors remained unaffected in the males (Barron, and Riley, 1985). The social play of the juvenile rat was not sexually dimorphic following prenatal exposure to etOH. While male offspring displayed feminized behavior, the female offspring displayed masculinized behavior (Meyer and Riley, 1986).

The above are representative effects of prenatal etOH exposure. Similar deficits, of a different intensity have also been observed following prenatal exposure to opiates.

MORPHINE AS A MODEL BEHAVIORAL TERATOGEN

Neonatal Opiate Withdrawal Syndrome

Opiates have rarely been reported to cause any malformations, when available prenatally. Such events are unlikely and require extreme levels of exposure (Harpel and Gautieri, 1968). It has been reported however that human infants born to opiate using mothers undergo mild withdrawal (Chasnoff and Burns, 1984). This "subacute withdrawal" phenomenon is a long lasting proccess extending from three up to five months. In particular, heroin consumed during pregnancy induced a non-specific arousal of neonatal human CNS activity. The gamut of symptoms includes hyperactivity, hyperexcitability, hyperacousis, sleeplesness, and prolonged high pitch-crying. The acute symptoms subside within 3-6 weeks, to be followed by subacute withdrawal symptoms, which persist for 4-6 months and include agitation, tremors, and sleep disturbances (Chasnoff, and Burns, 1984; Hutchings, 1985). The study of the neonatal withdrawal syndrome has been facilitated by different methods.

Models for the Study of Morphine Teratogenesis

The development of the neonatal withdrawal syndrome has been experimentally observed in the prenatal lamb (Szeto, Zhu, Amione, and Clare, 1988). The investigators confirmed the development of sleep-wake pattern disturbances, and a general hyperactivity during the final stages of gestation. Despite the specificity of the neonatal withdrawal syndrome to the later stages of gestation and the earlier stages of infancy, research has also been directed to other postnatal ages, for the assessment of any lasting morphine effects.

Behavioral and Developmental Effects of Prenatal Opiates

After reviewing existing evidence on the neonatal withdrawal syndrome Hutchings (1982) maintained that prenatal exposure to heroin, especially in a pattern of poly-drug use, can result in impaired organizational and perceptual abilities. In situations where motor inhibition was required, increased activity was obsereved. Sobrian (1977) had found that the induced hyperactivity was observed in the rat between the 3rd and the 4th postnatal weeks. Davis and Lin (1972) reported increased activity in the open-field at 1 and 2 1/2 months of age in the rat. Furthermore Sedlacek (1986) reported that the

chronic administration of opiate antagonists to chick embryos depressed the development of spontaneous chick motility to 26.1 - 75.8% of that of the controls. Both Sobrian (1977) and Davis and Lin (1972) reported a higher mortality rate and lower body weights only during the first week postpartum, with later measures being comparable to controls.

Similar to the effects of prenatal etOH on sexual development are the effects of prenatal opiate exposure. Vathy, Etgen, and Barfield (1985) reported a dimorphic effect of prenatal morphine on the sexual development of the rat. Although the female reproductive functions were disrupted, the male functions remained unaffected.

Most significantly however prenatal morphine and other opioids have been consistently reported to alter the tolerance of the organism toward stress, or toward the opiate effects when the latter are self- or forced-administered. Kirby, DeRosset, and Holtzman (1982) pointed out that the schedule of prenatal administration can determine the presence or the absence of enhanced postnatal tolerance to the analgesic effects of morphine. Wagner, Jarvis, Gottesfeld, Giorlando, and Rabii (1986) found that prenatal exposure to morphine, produced tolerance to the "response rate-disruptive" effects of acute morphine administration in the adult offspring. Earlier, Castellano and Ammassari-Teule (1984) had reported that in the

mice only activity measures were significantly reduced in morphine-exposed offspring, while postnatal reflexes involving motor control were only "slightly" deficient. Although there were no differences in baseline conditions between morphine- and saline-exposed offspring, enhanced responsiveness was evident towards morphine administration, and towards morphine administration and stress. Hovious, and Peters (1985) found that although prenatal (and perinatal) methadone administration did not induce postnatal methadone self-administration, it did result in a 75-80% preference for morphine solutions over water, in 85-90 day-old rat offspring placed on a self-administration schedule. The preference rates persisted after a drug-free period and were significantly higher than the rates observed in control animals.

At first glance etOH and the opiates seem to produce analogous developmental deficits, when available prenatally. But one needs to elaborate further on the relationship of etOH with opiates, in order to verify such an assumption.

ETOH AND OPIATES : THE STUDY OF THEIR INTERACTIONS

A relationship between ethanol and the opiates (especially morphine) has been often reported. The nature of the relationship however still remains unclear. Beaman, Hunter, Dunn, and Reid (1984) investigated the effects of morphine and naloxone (NX) on the alcohol consumption of water-deprived rats. Morphine was reported to increase the avidity for ethanol solutions, while its antagonist naloxone decreased the ethanol intake. Consideration of the historic evidence on the therapeutic role of opiates in alcoholism (Siegel, 1986), also verified the possibility of a relationship between agents. However the evidence reviewed by Siegel (1986) indicates a reversal of the avidity for etOH following administration of opiates. Satinder (1982), had offered additional support to the idea when he reported that if the genetic background of the organism is considered there seems to be a direct relationship between etOH and morphine preference. Specifically strains exhibiting a higher preference for etOH showed a similar preference for morphine. Conversely strains with a lower preference for morphine equally avoided solutions of etOH.

Because of the functional relevance of etOH and opiates the Endogenous Opiate System (EOS), has been repeatedly proposed as the model system to explain any etOH-opiate interactions. In 1970 Davis and Walsh had suggested that the tetrahydroisoquinolines (TIQs)- products of the etOH metabolism, may be responsible for the mediation of some etOH effects common with the opiates. According to Siegel (1986) this idea, though initially criticized, has more recently received "impressive support for the contribution of TIQs and endogenous opioids to the effects of etOH". In order to elaborate on the potential role of the EOS in the etOH-opiate interactions a brief description of the system is necessary.

The Characteristics of the EOS

Initial investigations in the area of opiate addiction and its treatment, verified the existence of the Endogenous Opiate System. The EOS is comprised of different types of receptors, which are unevenly distributed within the Central Nervous System (CNS). While there is general agreement about the opiate status of mu (μ), delta (δ), and kappa (κ) receptors, the evidence is only preliminary for the epsilon (ε), and the sigma (σ) receptors (Millan, 1986). Hammel and Baudet (1987) have reviewed evidence on the constitution of the EOS. The highest density of opiate-binding sites in the rodent occurs in the neostratum. In addition the d- receptors are concentrated

across the caudate putamen, and the μ-, and κ- receptors are mostly located within "islands or patches" that correspond to the terminal fields of prelimbic cortical projections, the vacancies in the termination of parafascicular projections, and acetylcholinesterase-poor striosomes (Hammel and Baudet, 1987). Mclean, Rothman, Jacobson, Rice, and Herkenham (1987) extended the findings for the hippocampus of the rat and identified the relative species differences between the squirrel, the guinea pig, the rat, and the hamster. Opiate peptides have also been identified in the human cerebrospinal fluid (CSF) (Cardinale, Donnerer, Fink, Kantrowitz, Oka, and Spector, 1987).

Because of the nature of the system -its products and functions- the role of the EOS in the development of addictions has been investigated. Several models, both in vivo and in vitro, have been developed to study the constitution of the EOS as well as the dynamics within the system. Most commonly used methods of study include either direct radioactive labelling of the EOS-related compounds or the study of their immunoreactivity. In addition behavioral studies have related the EOS activity with the behavioral outcome of dependence. In the following section both the physiological and the behavioral evidence on the role of the EOS in both opiate and ethanol addiction will be discussed.

EOS : the Locus for EtOH and Opiate Interactions

Physiological Studies

Balakleevski, Maslova, Petrenko, and Surikov (1986), found that after short-term exposure to etOH, the concentration of Leu-enkaphalins in the limbic cortex of the heavy drinking female rats was higher than in the control or the abstinent animals. The levels of Met-enkephalins were found to be less concentrated in the basal ganglia of the heavy drinking rats than in the controls and the abstinent animals. Long-term exposure on the other hand, left the Leu-enkephalines unaffected while the levels of Met-enkephalin dropped in the heavy drinking animals along with the levels of c-AMP, and c-GMP. Maizelis and Zabludovski (1986), showed that prenatal exposure to etOH results in a reduction of the levels of the Leu- and Met-enkephalin in the hypothalamus of the exposed rat.

There is also evidence of changes in the cerebrospinal fluid following opiate exposure, however no specific details are known of changes in the EOS (Olson,Olson, and Kastin, 1986). Interestingly and as is the case with etOH, the immune system is involved in the development of opiate dependence. Olson et al. (1986) in their review of relevant evidence reported that the "selective ablation of immunocompetent cells" suppressed all naloxone-precipitated signs of withdrawal. In addition the

administration of morphine and of the immunomodulator cyclosporine (or injection of spleen cells from animals that had received the same treatment) resulted in the suppression of withdrawal symptoms (Olson et al., 1986).

The evidence on the interactions of etOH and morphine within the EOS comes from different sources. A dramatic demonstration of the antagonistic influence of naloxone on the etOH-precipitated effects has been reported by Rae (1986). The condition of sixteen emergency patients admitted with etOH-induced comma improved shortly after the administration of naloxone. In addition Anokhina and Gamaleya (1988) reported the development of significant levels of antibodies to morphine in the blood serum of alcoholics.

Behavioral Studies of Preexposure

Evidence about the suspected actions of μ^1 receptors indicated that preexposure to etOH blocked morphine-induced Conditioned Taste Aversions (CTA's). Conversely preexposure to morphine blocked etOH-induced CTA's. The opiate antagonist naloxazone reversed the interaction between morphine and alcohol, when administered before the preexposure drug (Ng and Amit, 1985). According to the researchers the chronic ethanol exposure resulted in approximately similar conditions as did chronic morphine administration; i.e. the levels of endophrins

were decreased. Acute etOH administrations on the other hand resulted in significant increases in met-enkephalin levels in the hypothalamus, the striatum and the mid brain. This latter action suggests that etOH and morphine activated the opiate receptors and their synergistic effects produced the observed CTA's.

Reid and Hunter (1984), reported that increments in endogenous opiate activity increased the avidity of rats for etOH. When the antagonists naloxone and MR2266 were administered they protected against etOH-induced hypothermia, and enzyme activation. Tamborska Kotlinska, and Langwinski (1984), also presented analogous findings. The antagonists naloxone and naltrexone antagonized sleep and hypothermia induced by etOH. However the investigators suspected the involvement of other mechanisms additional to the EOS as the effects of etOH were antagonized by doses higher than the ones required to antagonize similar effects produced by morphine.

More specific evidence on the control of etOH motivation by induced alterations in the EOS was presented by Balakleevski et al. (1986). The investigators reported that effective anti-alcohol agents are to be found among the compounds with selective action on the activity of mu- and sigma-enkephalinergic brain structures.

It has been postulated that increased etOH ingestion folowing opiate administration may have been confounded with the effects of opiate agonists on water drinking. Spencer, Depuree, Hsiao, Mosberg, Hruby, Burks, and Porreca (1986) reported, that depending on the type of the introduced agonist and its selectivity to the receptor affected there is an effect on water drinking. The effect is also dependent on the efficiency of the agonist to bind the receptor. However prenatal exposure studies have established a definite relationship between the nature of the exposure (i.e. etOH or opiates),and the postnatal response towards the non-presented agent (e.g. opiates or etOH).

Studies of Prenatal Exposure and Postnatal Development

In a series of experiments, the effects of Fetal Ethanol Exposure (FEE) were studied in terms of their interaction with a presented stressor (Taylor et al., 1984). Acute presentation of prolonged/intermittent footshock caused an opiate mediated analgesia that was potentiated in FEE animals. Similarly chronic presentation of the same stressor resulted in perturbations of the EOS that were more prominent in the animals prenatally exposed to etOH.

Nelson, Taylor, Lewis, and Branch (1986), and Nelson, Taylor, Lewis, Russel, Reid and Branch (1986) noted that rats prenatally exposed to ethanol, showed significantly higher corticosterone levels than the control animals, following

morphine administration. Furthermore the etOH-exposed animals were also hyperresponsive to intermittent but not to continuous footshock. Neil, Kayser, Gacel, Bessonand, and Guilland (1986), extended the findings by indicating that Fetal Ethanol-Exposed (FEE) rats were hypersensitive to the analgesic and pituitary-adrenal activating effects of morphine. In addition Nelson, Lewis, Kokka, Branch,and Taylor (1986) noted that the etOH-induced hypothermic responses were also potentiated by the administration of 10 and 30 mg/kg postnatal doses of morphine. It is therefore possible that postnatal etOH effects lead to permanent perturbations in the Opioid System and their consequences appear as changes in the postnatal behavior.

The reviewed evidence points towards the existence of a relationship between etOH and the opiates. This relationship, synagonistic in nature has been confirmed in a variety of situations. The reviewed evidence also indicates a significant role of the EOS in the expression of this interaction.

There have been no efforts to investigate the effects of concurrent administration of etOH and opiates. Similarly the study of the effects of postnatal exposure has been limited to the administration of one of the agents at a time. In order to understand fully the nature of the interaction of etOH and the opiates, one needs to employ the concurrent administration of the two agents as a standard control procedure.

The role of the development of the EOS in the expression of the interactions between etOH and opiates following prenatal exposure has not been clearly shown. We therefore need to describe that role through the effect of prenatal exposure on the behaviors most likely to be affected. In order to resolve some of the existing issues and also to explore possibilities for the improvement of the existing methodologies the following design was arrived at.

Rationale of the Present Study

The present study was designed to investigate the effects of prenatal etOH and morphine, as well as their combination, on the postnatal development and behavior of the neonatal rat. The effects of self-administration of etOH, and/or opiates were investigated within the behavioral teratological paradigm. It has been suggested that behavior can be a more sensitive indicator of central nervous system toxicity than morphology, provided that the employed behavioral methods are valid and sensitive to the effects of the chemical exposure (Norton, 1978).

The behavioral tests used in this study (Either-way Avoidance, Reactivity testing, and Hot-PLate Analgesia) have been previously used and reported efficient for the study of the effects of prenatal chemical exposure (Zagon, and McLaughlin, 1986; Zbinden, 1981). A test battery was also adopted to identify the changes in the early development of the offspring due to the prenatal exposure. There has been no previous attempts however to formalize such a procedure. In order to develop such a procedure a test battery previously used in our laboratory was employed (Pelletier, 1988). The procedure measured the physical, sexual, and reflex development, and the emergence of spontaneous behavior (crawling and walking). The characteristics of the test battery items are outlined in Appendix I.

In order to avoid any bias on the part of the experimenter the standardized procedures of double blind controls were employed. All the dams and their offspring were randomly coded in a way so that the experimenter was unaware of the exposure they had received.

METHOD

Animals and Prenatal Exposure

All the animals used in this study were experimentally naive, selected from the stock existing in the Psychology Laboratory at Lakehead University. The study involved the use of 36 animals for the necessary pairings (i.e. at least 3 pairings per cell of prenatal condition). The 18 male and 18 female animals were selected from the Satinder Heterogeneous Stock (SHS). The number of offspring tested were 116 animals.

The SHS line has been developed through a four-way cross of the Roman high avoidance (RHA), Roman low avoidance (RLA), Maudsley reactive (MR), and Maudsley non reactive (MNR) genetic lines (Satinder, 1980). The RHA and RLA genetic lines have been subjected to genetic selection for high and low rates of two-way active avoidance respectively. Detailed descriptions of the RHA and the RLA lines can be found in Bignami (1965). The MR and the MNR lines have been respectively subjected to genetic selection for high and low defecation in the open field. The characteristics of the Maudsley lines have been discussed by Broadhurst (1975).

All animals were maintained under the standard laboratory conditions of light, and temperature. Specifically all rooms were maintained on a 12/12 hr light/dark cycle (with lights on

at 08:00 EST). The temperature fluctuated between 22 ± 2°C, and the humidity level was maintained at 40%. Food and water were freely available unless otherwise prescribed by the procedure.

Procedure

Prenatal Exposure

Female animals selected for mating were littermate quintuplets, from the SHS strain, and were allowed a one week period to adjust in the setting of the pairing room. During that time baseline measures of body weight, food and water consumption were taken. The animals were individually housed in standard wire-mesh cages. Following the baseline period and before the start of the prenatal exposure the female animals were paired with male siblings from different litters. The detection of a vaginal plug served as the indication of the beginning of pregnancy (gestation day 0). At that point the male animals were removed and the prenatal condition was initiated. The female animals were matched for body weight and assigned to one of the following conditions:

1) Ingestion of a .5 mg/ml morphine solution, as the only fluid available.

2) Ingestion of a 10%(v/v) etOH solution, as the only fluid available.

3) Ingestion of a cocktail of .5 mg/ml morphine and 10%(v/v)

etOH solution, as the only fluid available.

4) Ingestion of a cocktail of .25 mg/ml morphine and 5%(v/v) etOH solution, as the only fluid available.

5) Ingestion of water, as the only fluid available.

In order to increase the palatability of the etOH and morphine solutions, and in order to equalize any effects due to the differential caloric contents of these solutions two types of sweeteners were added. A pilot investigation was conducted to ensure that the consumption rates were equal for the different solutions. In addition an effort was made to ensure that the levels of fluid consumption remained above the minimum daily requirements of drinking. These requirements were met by the solutions described above. Saccharin (the non-caloric sweetener) was used for the etOH solution, the .5 mg/ml morphine-10% etOH cocktail and the .25 mg/ml morphine-5% etOH cocktail. Sugar (3.9 cal/gr)was added to the morphine solution, and the .25 mg/ml morphine-5% etOH cocktail, up to an amount neccessary to match the calories contained in an equal volume of the 10% etOH solution. The saccharin was added at a rate equivalent of one 1/4 grain per 5 grams of sugar.

Daily recordings of body weight, food and fluid intake were taken from gestation day 0 and until gestation day 21. The female animals were then transfered to the breeding room, and allowed to litter. All dams and their offspring were housed in standard "shoebox" breeding cages until weaning (Day 28

postpartum). At weaning (day 28 postpartum) all offspring were transfered in the experimental room and individually housed in standard wire mesh cages. Any offspring that died before the end of the procedure were preserved in formaldehyde, and were later observed for morphological aberrations.

Experimental Design

The prenatal component of the design involved 5 experimental conditions. At least three live litters per treatment were included for a total of 18 litters. To study the progress of pregnancy and conduct the analyses between conditions, the means of the dams from each treatment were included as the relevant experimental units. In the early stages of the study it became evident that at least two (i.e. the etOH, and the high dose of etOH-morphine) of the prenatal exposures had a detrimental effect in either the completion of full-term pregnancy, or the survival of the litters. However the design of the study permitted only the study of viable offspring. To circumvent the problem the original criterion of three pairings per treatment was adjusted to at least three live litters per prenatal treatment. This was done in order to satisfy the theoretical requirements of the employed design. The data on the survival of the offspring are important in a teratological design since the teratologist is interested in the effects which limit the survival (viability) or affect the

appearance of the offspring. The behavioral teratologist however is interested in quantifying the effect of the prenatal insult that has resulted in behavioral deficits.

Hutchings (1985) has confirmed that the effect of the prenatal treatment extends also after birth. The new-born offspring depends on the dam for its food supply and thermoregulation. However following weaning the strength of the uterine and perinatal influences dissipates. Since the offspring remain independent from the maternal influence they can be considered individually as units for our analyses. As Suter and Schon (1986) have suggested this increase in sampling units, might also facilitate the interpretation of the effects of prenatal exposure, especially on learning.

Given the above considerations the resulting postnatal design was a 5 (prenatal conditions) X 2 (sexes) factorial.

Apparatus

Negative Geotaxis

An incline plane of 30 was constructed from a 20cm square clear Plexiglas sheet. The sheet was elevated 12.5cm on one side in order to provide the necessary angle and was then permanently glued between two Plexiglas sheets affixed on a

right angle. The surface of the incline had been scored with sand paper and knife to provide adequate grip. A reference start line was drawn in the centre of the incline and parallel to the horizontal plane.

Unconditioned Escape Response (UER) and Avoidance Apparatus

Unconditioned Escape and Avoidance were tested in a circular Plexiglas runway (12 cm wide, and 15 cm high, with an outside circumference of 220 cm). The runway was divided into four compartments separated by guillotine doors, and was constructed of stainless steel rods, 0.25 cm in diameter, spaced 1 cm apart. The shock unit powered by 115 Volts AC, had a step-up transformer with a secondary rating of 3,000 Volts (Hammon Mode 216-60). The shock was delivered through a 2.7 X 1,000,000 Ohm resistor, assuming an additional animal resistance of 47,000 ohms (Satinder, 1976). Automatic timers were used to control the duration of the electric shock. A digital clock was used to record the response latencies.

The UER is genetic-line dependent and according to Satinder (1976) the existing differences can affect avoidance behavior. The procedure of establishing the UER was employed in order to avoid any differences in learning due to the genetic extremities inherent in the SHS strain.

Hot-Plate

A Hot plate (Model 60 from Force Electric Products Ltd., Acton, Ontario) was used, in conjunction with a "Powerstat" variable autotransformer (model 116B from Superior Electric Co., Bristol, Conn., USA), to control the required 53° C temperature in the centre of the hot plate.

Postnatal Procedures

Preweaning Measures of Development

The following test battery has been adapted from Jensh (1981).

The physical development of the litters was assessed by weekly body weight measures and daily examination for pinna detachment, incisor eruption, primary coat, eye opening, and descent of testes or vaginal opening.

The development and the integration of the motor and the vestibular systems were assessed by daily examination of the surface righting and the negative geotaxis reflexes.

The development of spontaneous behaviors (i.e. ambulation without aversive or other stimulation) was assessed by daily examination for crawling and walking. The reader is referred to Appendix I, for the criteria describing each characteristic, as

well as the beginning of its assessment.

Postweaning Development and Preference Testing

Following weaning and for one week (days 29-35 postpartum) all littermates were tested for water and food consumption, body weight development, and ethanol-morphine preference. Specifically, on days 31, 32, and 33, water was replaced by etOH (or morphine), morphine (or etOH), and etOH and morphine solutions respectively. An individual preference rate was calculated as the ratio between the total and the etOH (or morphine) consumption on day 33.

Reactivity Testing

On day 35 all littermates were allowed a 1 min exploration period in the avoidance apparatus. The animals were observed for latency to begin exploration, amount of activity, and defecation. This phase replaced the traditional reactivity testing in the open-field.

Unconditioned Escape Response

On day 36 littermate offspring were tested for UER. During this testing phase the experimental room lights were kept on. Every animal was allowed 1 min to adapt in the runway prior to the beginning of the session. The guillotine doors were adjusted to the height of the animal, so that the escape response could be associated with salient cues. Consequently an electric foot shock was administered for 5 sec or until the animal ran a distance equivalent to a quarter length of the runway in either direction (and this was defined as UER). When the animal failed to escape two consecutive times the setting was adjusted to the next higher level until a UER was obtained. Each animal was given 10 trials, with an intertrial interval of 5 seconds. Using the method of limits: ascending series, the UER was established as a shock intensity ranging between 0.1-0.9 mA, in 9 steps. The highest electric shock intensity required to elicit UER for each animal was used as the unconditioned stimulus for avoidance training.

Either-way Avoidance Learning

Starting on day 38 postpartum all animals were tested for avoidance learning. During this testing phase the lights of the experimental room were kept off. Only a 25 Watt shielded lamp was used as light source for the illumination of the instrument panel and for recording the data. The UER for each animal was used as an Uncoditioned Stimulus (US) during their avoidance

training. The Conditioned Stimulus (CS) was a 70 db 90 Hz noise preceeding the onset of the US by 10 seconds. Each animal was required to perform an avoidance response (i.e. run a distance equivalent to a quarter length of the runway before the onset of the US in either direction) was sufficient for the completion of a trial. When an avoidance response did not occur the CS and the US remained present until a total time of 20 seconds had elapsed, or until an escape response (i.e. a distance equivalent to a quarter length of the runway ran after the onset of the US in either direction) had occured. At the end of each trial the experimenter recorded either an avoidance, or an escape, or a non- response. The first response on the first trial of the first day was required to be an escape. To satisfy the condition of the particular trial, only two adjacent sections of the runway were available to the animal. Following the first trial and for the reasons described before, all the guillotine doors were adjusted. The latency to respond, the type of the response (avoidance or escape) and its direction (towards or away from the compartment previously occupied), as well as the intertrial crossings were recorded. Each subject was given four daily sessions of ten trials, with an intertrial interval of 40 seconds.

Whenever the animal responded to the CS by turning away from the compartment where it previously received a shock, the response was classified as a One-way avoidance. An avoidance response towards the compartment where the shock was previously received was classifed as a Two-way avoidance. The sum of

One-way and Two-way avoidance responses was described as the Either- way avoidance score. According to Satinder (1977) the animals have to attend to several cues in order to attain the required response while in the runway. While a One-way response simply requires the animal to run away from the compartment previously occupied, the Two-way response requires the animal to return to the compartment in which it was previously shocked. The cues that need to be attended are different in both cases. Comparatively the Two-way response is the more complex of the two tasks, as it requires the most cues to be attended to.

Analgesia Testing

On day 43 postpartum all littermates were tested for analgesia in a hot-plate situation. The testing consisted of 10 trials. The animals were placed on the hot plate and their latency to escape was recorded. In order to determine the optimal temperature (53°C) on the centre of the hot plate a pilot investigation was conducted utilizing 3 different temperatures. Traditionally the hot plate has been used in the study of opiate analgesia following one-time administration of morphine. The same method has also been recommended by Zagon and Mclaughlin (1982) as a way to assess some of the effects of prenatal morphine that influence young and adult animals.

RESULTS

The statistical analysis on the data was performed with the factorial analysis of variance (ANOVA). One-way ANOVA with the Scheffe Test was used for the requisite post-hoc analyses. The MANOVA procedure with repeated measures was used to analyze the Avoidance responses. For general purposes differences with associated probabilities less than 0.01 were considered significant. However, probabilities higher than 0.01 and less than 0.05 were reported to provide a better perspective. The aforementioned statistical procedures were performed with the SPSSx statistical package available on the Microvax II mainframe computer at Lakehead University.

Reproductive Indices

During the week preceeding pairing and prenatal exposure, there were no differences in the measures of body weight, food, and water consumption between the females exposed to the different experimental conditions. The measures from the pregnant females were analyzed on a daily basis. For purposes of easier reference the above measures were also analyzed on a trimester basis. The latter results will be reported here. Data for these measures are presented in Figures 1, 2, and 3.

During the first trimester of pregnancy (gestation days 1-7) there were no significant differences in body weight changes due to prenatal exposure. However there were significant differences in fluid consumption due to prenatal exposure ($F(4,16)=11.25$, $p<0.002$). The Scheffe comparison of the means revealed that the dams exposed to water consumed more fluid than the dams exposed to either etOH, or morphine, or etOH-morphine (low), or etOH-morphine (high). Similarly significant were the differences for food consumption due to prenatal exposure ($F(4,16)=5.64$, $p<0.005$). The Scheffe comparison of the means revealed that the dams exposed to water consumed more food than the dams exposed to either morphine, or etOH-morphine (high).

During the second trimester (gestation days 8-14) significant body weight differences due to prenatal exposure were evident ($F(4,16)=6.20$, $p<0.0033$). According to the Scheffe comparison of means the dams exposed to water weighed more than the dams exposed to either etOH, or etOH-morphine (high), or etOH-morphine (low). The differences in fluid consumption due to prenatal exposure remained significant during this period ($F(4,16)=3.99$, $p<0.01$). However no significant differences between group means were identified by the Scheffe test. Significant differences due to prenatal exposure for food consumption were also evident ($F(4,16)=11.42$, $p<0.0001$). The comparison of means with the Scheffe test revealed that the dams

exposed to water consumed more food than the dams exposed to either etOH-morphine (high), or etOH, or etOH-morphine (low), or morphine.

Figure1a. Maternal Body Weight vs Pregnancy Trimester

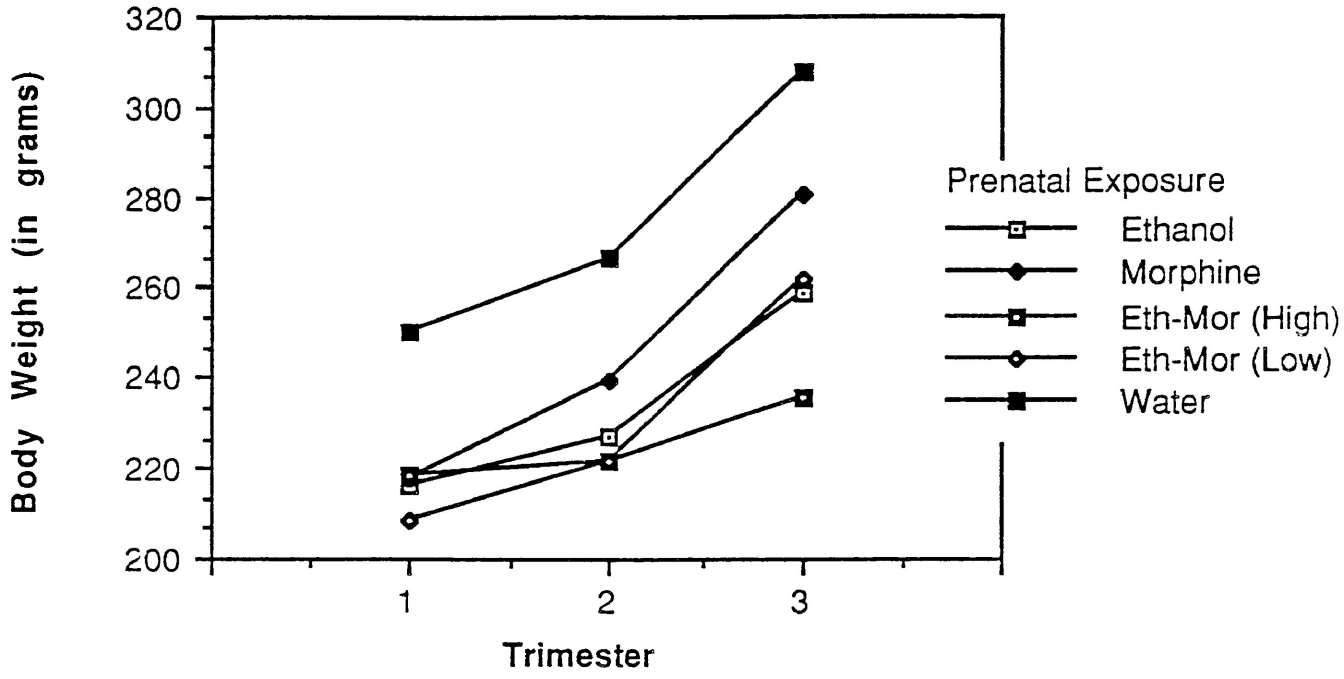
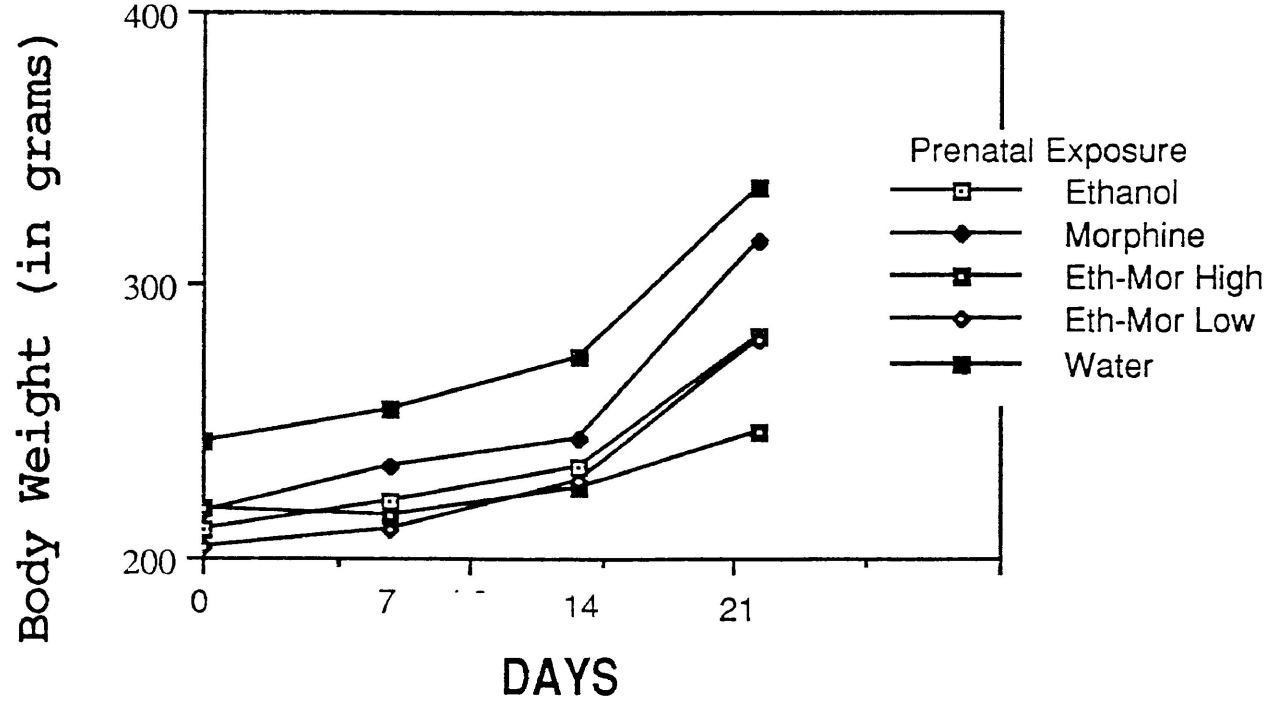


Figure 1b. Body Weight During Pregnancy (weekly)



During the third trimester (gestation days 15-21) the differences in body weights due to differential prenatal exposure remained significant ($F(4,16)=10.53$, $p<0.0002$). Differences due to prenatal exposure were significant for fluid consumption ($F(4,16)=14.22$, $p<0.00001$). Differences in food consumption due to prenatal exposure were also significant ($F(4,16)=14.35$, $p<0.00001$), in the third trimester. The comparison of means with the Scheffe test indicated that the dams exposed to water had higher body weights, consumed more food, and consumed more fluid than the dams exposed to etOH, or morphine, or etOH-morphine (high), or etOH-morphine (low).

Significant differences due to prenatal exposure were also observed for percentage of body weight gain throughout pregnancy ($F(4,16)=4.56$, $p<0.01$). However no significant group differences were reported through Scheffe's comparison of means.

There were no differences due to prenatal exposure in the length of gestation. There were no significant differences due to prenatal exposure in litter size, at birth and at weaning, or mortality of pups and other measures of reproductive success. The data listed in Table 3 however, reveal a differential toxicity among prenatal treatments. For example 60% of both morphine- and etOH-morphine (low)-exposed pregnant females, 71% of pregnant etOH-exposed females, 75% of the etOH-morphine

(high)-exposed, and 80% of the water-exposed pregnant females completed the full term of pregnancy.

The highest survival rate of litters to weaning was observed in the water-, the morphine-, and the etOH-morphine (low)-exposed groups (100%). Of the etOH-exposed litters 80% survived to weaning, and 66% of the etOH-morphine (high)-exposed litters were weaned. The overall mortality rate, from birth to weaning, was lowest in the water- exposed group, 91% of the pups born were weaned. The same measures in the other conditions ranged from 55% (etOH), to 65% (etOH-morphine (high)), to 69% (morphine), to 75% (etOH-morphine (low)). A higher incident of mortality was also apparent among males than females. Though none of the described trends were found significant, the importance of such observations should not be underestimated by the lack of significance. It should be noted that the increased mortality among conditions produced small samples for the relevant comparisons, and influenced the estimates of significance.

Figure 2. Maternal Fluid Consumption vs Trimester

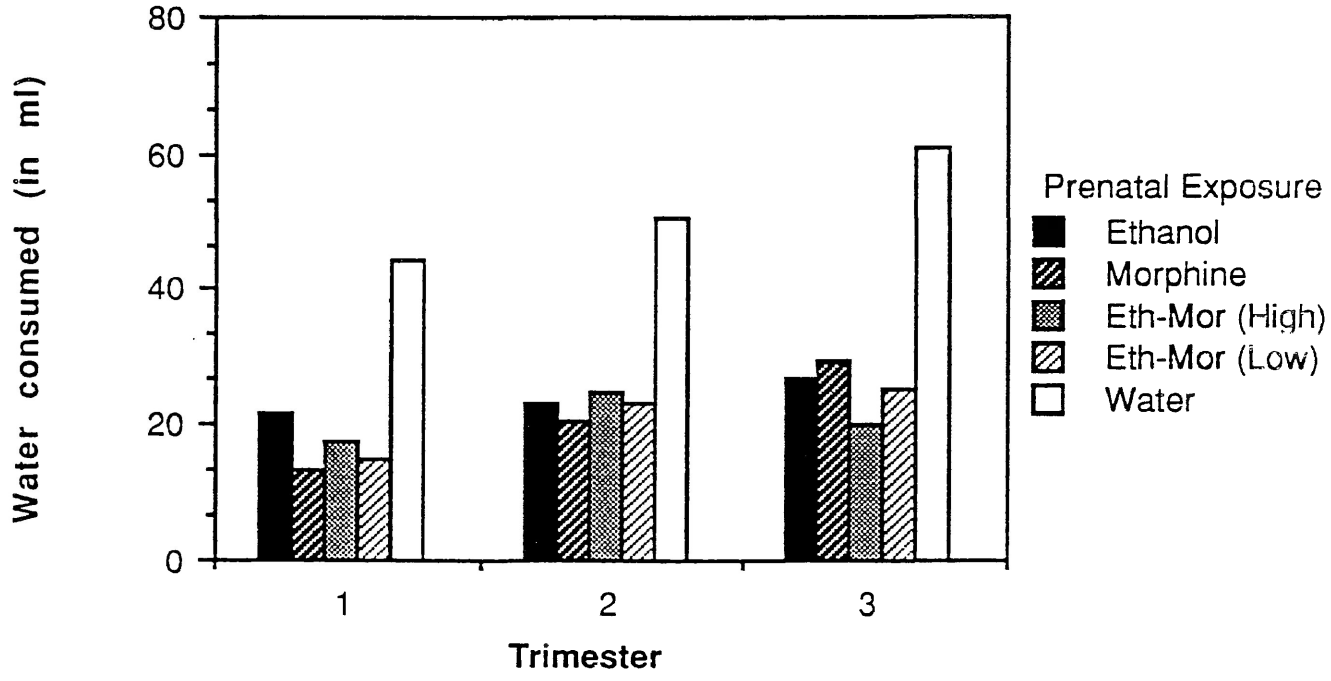


Figure 3. Maternal Food Consumption vs Trimester

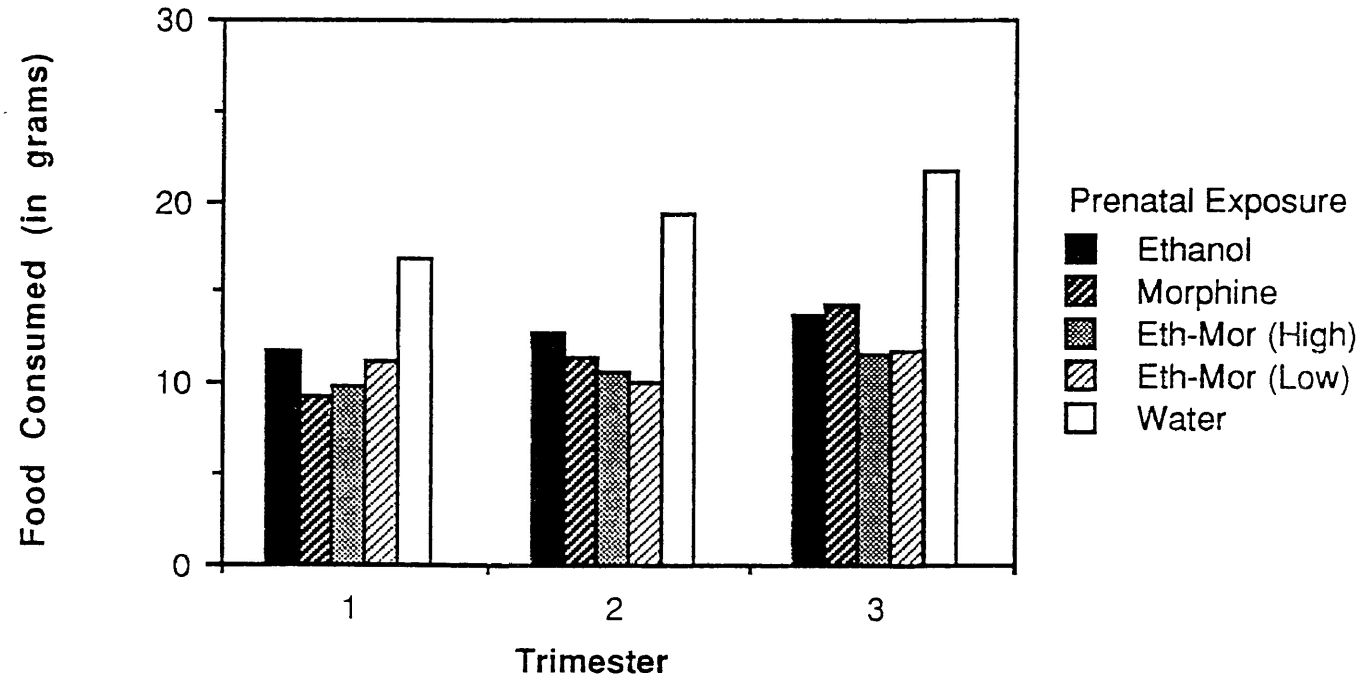
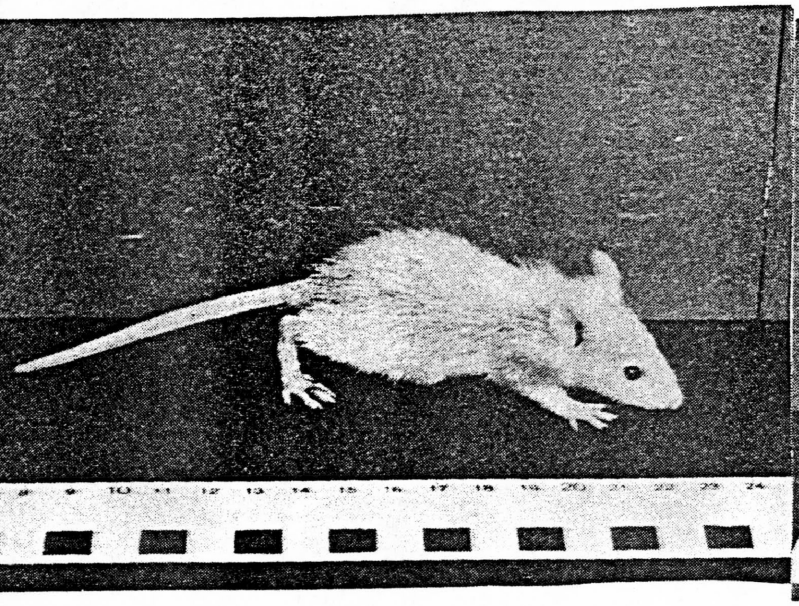


Table 3. Reproductive Indices

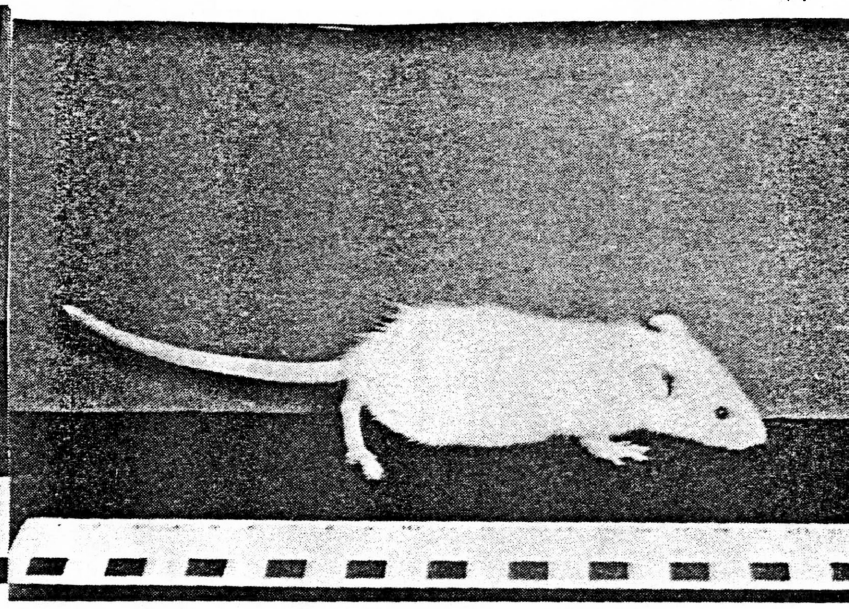
Pren.Exposure	Ethanol	Morphine	Eth-Mor Low	Eth-Mor High	Water
Total					
Females pair.	7	5	5	8	5
Females impregnated	7	5	5	8	5
Females littered	5	3	3	6	4
Litters Wean.	4	3	3	4	4
Mean(with SD)					
Number of Gestation days	22.40 (0.54)	22.66 (0.57)	22.66 (0.57)	23.00 (0.00)	22.75 (0.50)
Pup number at birth	6.80 (3.83)	11.00 (3.46)	9.33 (2.08)	6.40 (3.20)	8.75 (2.87)
Male pups at birth	4.20 (3.11)	5.00 (2.00)	5.00 (1.73)	4.00 (1.58)	4.25 (1.25)
Female pups at birth	2.60 (1.94)	6.00 (2.00)	4.33 (2.30)	2.40 (2.07)	4.50 (1.73)
Males Born/ Females Born	1.61	0.83	1.15	1.66	0.94
Pup number at weaning	3.80 (3.34)	7.66 (0.57)	7.00 (1.00)	4.20 (3.27)	8.00 (2.94)
Male pups at weaning	2.00 (2.73)	3.33 (1.52)	3.66 (1.52)	2.40 (1.51)	3.75 (1.50)
Female pups at weaning	1.80 (1.64)	4.33 (1.52)	3.33 (2.30)	1.80 (2.04)	4.25 (1.70)
Males / Females Wean.	1.10	0.76	1.09	1.30	0.88
Pups Weaned/ Pups Born	0.55	0.69	0.75	0.65	0.91
Mal. Weaned/ Males Born	0.47	0.66	0.73	0.60	0.88
Fem. Weaned/ Fem. Born	0.69	0.72	0.76	0.75	0.94
Mortality rate (Mean)	3.00 (2.55)	3.33 (2.88)	2.33 (1.15)	2.20 (1.92)	0.75 (0.95)

On two instances the teratogenic effects of ethanol and etOH-morphine (high) on the development were clearly visible in the surviving offspring (for comparisons refer to Figure 4). It was therefore thought necessary to account in a complete manner for the two offspring identified as dysmorphogenic. These comparisons revealed a possible protective influence of morphine, from the effects of etOH. Of the two males exhibiting delayed growth, the animal exposed to etOH-morphine (high) was only deficient in body length and weight. The animal exposed to etOH only exhibited both delayed body and weight growth and deficient development of fur. These differences were evident for both offspring until they were sacrificed (on day 44). No other teratogenic effects were observed in either the live or the dead offspring.

Considering the differences in the teratogenic action, and the differences in the rates of mortality between etOH and etOH-morphine (high) exposed offspring one might risk the assumption that there appears to be a protective morphine influence from some of the effects of etOH. The importance and the validity of this assumption are presented at a later section.



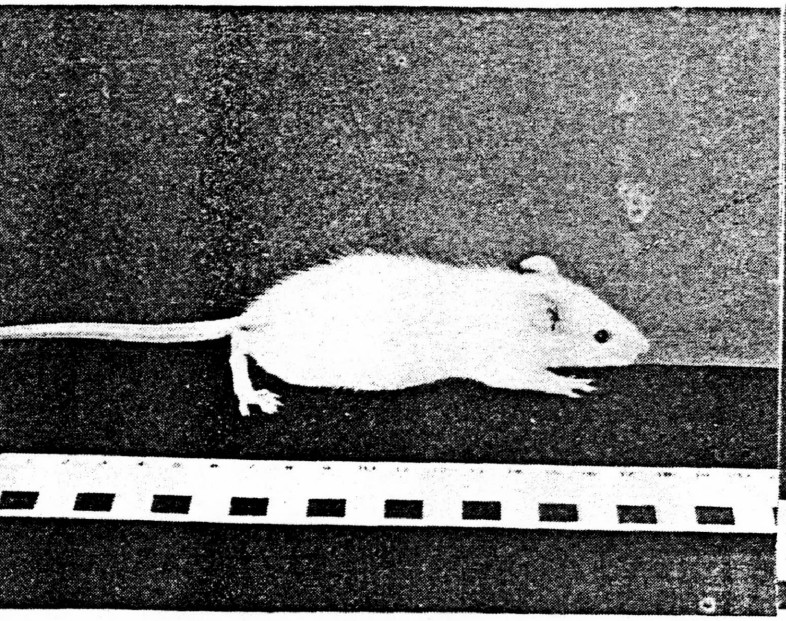
etOH-exposed offspring exhibiting delayed growth (male)



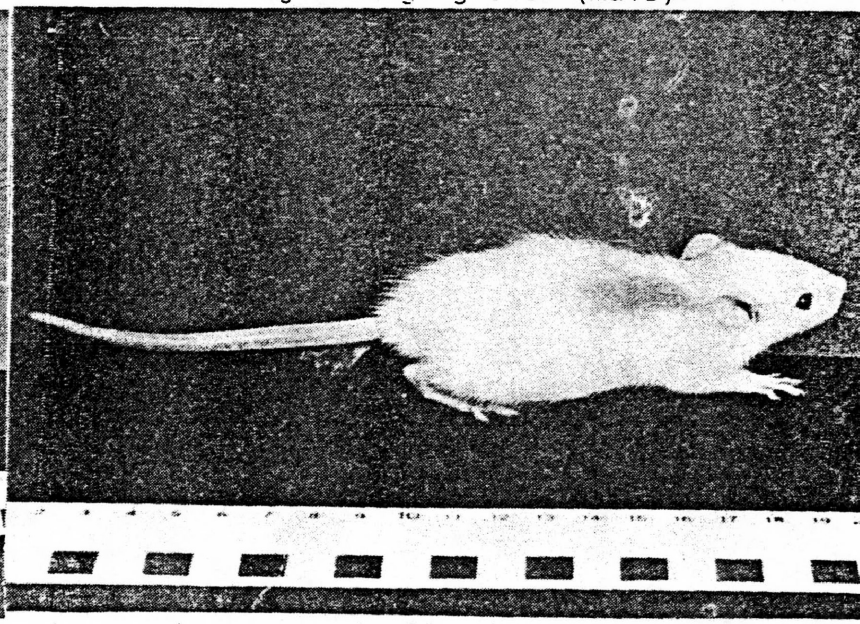
etOH-exposed offspring exhibiting average growth (male)

etOH-morphine (high)-exposed offspring exhibiting delayed growth (male)

etOH-morphine (high)-exposed offspring exhibiting average growth (male)



water-exposed offspring exhibiting average growth (female)



water-exposed offsoring exhibiting average growth (male)

Developmental Indices

Body Weight Measures

There were no significant interactions between prenatal exposure and sex on body weight change. There were however significant body weight differences due to prenatal exposure that emerged on day 7, and remained until day 41. The differences disappeared on day 43, the last day at which body weights were recorded. The lowest differences in body weight due to prenatal exposure were observed on day 41 ($F(4,112)=3.49$, $p<0.01$). The Scheffe comparisons of the means revealed the following relationships:

1)the water-exposed offspring weighed more than: the etOH-exposed offspring on days 29, and 32-34; the morphine-exposed offspring on days 7,29, 34-37, and 41; the etOH-morphine (high)-exposed offspring on days 21, 28, 29, 30, and 32-36.

2)The etOH-morphine (low)-exposed offspring weighed more than the etOH-exposed offspring on days 28-35; the morphine-exposed offspring on days 14, 28-30, and 34-39; the etOH-morphine (high)-exposed offspring on days 14, 21, and 28-36. The etOH-exposed offspring weighed more than the morphine-exposed offspring on day 7.

The data on body weight changes are summarized on Tables 4,and 5. No differences were found in body weights between the water-

and the etOH-morphine (low)-exposed offspring. Rather the two groups exhibited a similar pattern of body weight changes.

Significant differences in body weight between males and females emerged on day 31 ($F(1,115)=8.98$, $p<0.003$). There were no significant body weight differences found between sexes on days 32, and 33. However the body weight differences between males and females re-emerged from day 34, onwards. The minimum difference in male and female body weights occured on day 34 ($F(1,115)=8.89$, $p<0.0035$) (see Table 5).

The analysis of body weight measures confirmed the findings reported by Nathaniel et al (1986), and Abel and Dintcheff (1978). The etOH-exposed animals exhibited an overall body weight growth delay, with postnatal "catch up", from day 36, onwards, while the offspring exposed to etOH-morphine (high) were affected to a greater extend. The postnatal catch-up growth, for instance, was delayed until day 39.

The exposure to morphine resulted in an extensive delay of growth, atypical according to the existing reports (Sobrian, 1977; Davis, and Lin, 1972). The body weight growth for this group appeared to be delayed as early as day 7, and the process of catch-up growth was not evident until day 43.

An interesting reversal in body weight development occured during the days of drug-preference testing (i.e. days 31, 32, 33). Namely the morphine-exposed offspring exhibited growth that was not significantly different from the water-exposed offspring. The data suggest that postnatal exposure to morphine may possibly reverse some of the developmental deficits caused by the prenatal experience.

It was suggested earlier, that the prenatal presence of morphine protected the offspring from some of the teratogenic effects of etOH. A reverse influence was also observed. Namely the prenatal presence of etOH in both the etOH-morphine (high), and etOH-morphine (low) groups seemed to have protected the offspring from the effects of morphine on body weight development, and the appearence of developmental landmarks, as it will become apparent in the following section.

Physical Development

All data from the test battery are summarized and presented on Table 6. There were no significant differences due to prenatal exposure for pinna detachment.

Table 4. Mean Body Weight (with SD) of Offspring By Prenatal Exposure

Pren.Exposure	Ethanol	Morphine	Eth-Mor High	Eth-Mor Low	Water	Signific. Level
Mean(with SD)						(1WayANOVA)
DAY 1 (postpartum)	6.47 (0.84)	6.08 (1.12)	6.13 (0.71)	6.38 (0.74)	6.78 (0.83)	
DAY 7 (postpartum)	14.10 (1.79)	*@12.04 (2.38)	13.13 (1.72)	13.71 (2.10)	13.81 (1.51)	(p<0.00330)
DAY 14 (postpartum)	25.00 (2.47)	^23.26 (3.69)	^24.09 (3.32)	27.52 (3.23)	25.34 (2.93)	(p<0.00040)
DAY 21 (postpartum)	38.89 (3.39)	38.95 (5.54)	*^35.54 (7.06)	41.23 (5.01)	40.56 (5.28)	(p<0.00660)
DAY28 (postpartum)	^57.31 (7.22)	^57.95 (7.44)	*^51.40 (11.16)	68.57 (6.30)	64.21 (6.63)	(p<0.00001)
DAY 29 (postpartum)	*^61.47 (5.78)	*^62.26 (8.00)	*^59.31 (7.04)	69.95 (7.49)	68.21 (6.00)	(p<0.00001)
DAY 30 (postpartum)	^66.00 (5.72)	^66.34 (8.61)	*^63.31 (7.28)	74.28 (7.34)	71.84 (6.24)	(p<0.00001)
DAY 31 (postpartum)	^63.47 (7.43)	68.82 (7.15)	^65.04 (8.70)	75.09 (9.76)	70.90 (9.58)	(p<0.00020)
DAY 32 (postpartum)	*^65.10 (7.27)	70.26 (10.42)	*^65.40 (7.50)	75.95 (9.28)	73.40 (9.35)	(p<0.00001)
DAY 33 (postpartum)	*^69.63 (6.98)	75.82 (9.98)	*^71.45 (7.30)	82.23 (9.24)	78.84 (8.29)	(p<0.00001)
DAY 34 (postpartum)	*^77.57 (7.50)	*^78.04 (10.92)	*^74.27 (7.40)	86.42 (8.29)	85.31 (7.35)	(p<0.00001)
DAY 35 (postpartum)	^82.78 (6.45)	*^82.17 (10.89)	*^81.31 (7.35)	93.47 (8.49)	90.43 (8.20)	(p<0.00010)
DAY 36 (postpartum)	88.26 (7.35)	*^87.21 (12.09)	*^87.27 (8.07)	96.57 (9.88	95.68 (8.52)	(p<0.00020)
DAY 38 (postpartum)	98.52 (7.69)	*^96.52 (13.61)	97.95 (10.00)	108.42 (11.26)	106.31 (10.93)	(p<0.00030)
DAY 39 (postpartum)	103.68 (7.60)	^100.78 (14.27)	^101.59 (10.12)	113.19 (11.51)	110.21 (10.59)	(p<0.00030)
DAY 40 (postpartum)	108.05 (8.40)	104.13 (15.14)	107.09 (10.65)	114.76 (11.66)	113.62 (11.06)	(p<0.00870)
DAY 41 (postpartum)	111.73 (9.11)	*107.26 (15.60)	111.27 (10.54)	117.47 (14.04)	118.75 (12.54)	(p<0.01000)
DAY 43 (postpartum)	119.31 (10.32)	116.17 (17.02)	120.00 (11.87)	124.81 (18.60)	127.37 (14.71)	(p<0.00500)

@:Ethanol > the designated group mean

*:Water > the designated group mean

^:Eth-Mor Lo> the designated group mean

Table 5. Mean Body Weight (with SD) of Offspring By Sex

Column 1 Mean(withSD)	Female	Male	Signif. Level (1WayANOVA)
DAY 1	6.25 (0.93)	6.54 (0.82)	
DAY 7	13.17 (2.07)	13.54 (1.93)	
DAY 14	24.75 (3.80)	25.29 (2.99)	
DAY 21	38.76 (5.49)	39.50 (5.87)	
DAY 28	58.91 (10.39)	61.45 (8.91)	
DAY 29	63.50 (7.38)	65.59 (8.27)	
DAY 30	67.10 (7.46)	70.06 (8.34)	
DAY 31	66.30 (7.83)	71.36 (10.14)	(p<0.00330)
DAY 32	68.69 (9.84)	71.95 (9.43)	
DAY 33	74.10 (8.61)	77.68 (9.90)	(p<0.03980)
DAY 34	78.10 (8.76)	83.18 (9.57)	(p<0.00350)
DAY 35	83.42 (8.45)	89.13 (9.95)	(p<0.00120)
DAY 36	87.87 (8.81)	94.62 (10.23)	(p<0.00020)
DAY 38	97.30 (8.85)	106.18 (12.66)	(p<0.00001)
DAY 39	101.32 (9.14)	110.70 (12.54)	(p<0.00001)
DAY 40	103.87 (8.91)	115.29 (12.23)	(p<0.00001)
DAY 41	106.87 (9.67)	120.00 (13.10)	(p<0.00001)
DAY 43	113.28 (10.76)	130.03 (14.38)	(p<0.00001)

Table 6. Appearence (with SD) of Developmental Indices, in Days

Pren.Exposure	Etl	Morphine	Eth-Mor Low	Eth-Mor High	Water	Column 7
Mean(with SD) of appearence						Siginif. Level (1WayANOVA)
Surface righting	.10 .64	4.60 2.36	# 2.90 0.83	# 3.14 0.77	# 2.81 0.59	(p<0.00001)
Pinna detachment	.00 .72	3.13 0.86	2.57 0.50	2.74 0.52	2.75 0.44	(p<0.02000)
Incisor eruption	.65 .23	* 9.87 1.14	11.76 1.30	* 10.37 0.83	* 9.87 1.23	(p<0.00001)
Primary coat	.00 .00	8.34 0.48	8.33 0.48	#*8.00 0.00	8.21 0.42	(p<0.00070)
Crawling	.50 .82	7.78 1.12	# 7.14 0.65	# 7.11 0.32	# 7.00 0.00	(p<0.00020)
Walking	.30 .10	15.47 1.53	14.85 1.45	15.77 1.28	#*^ 12.93 1.72	(p<0.00001)
Negative geotaxis	.35 .74	11.00 1.16	# 10.09 0.43	10.33 0.83	10.40 0.56	(p<0.00330)
Eye opening	.05 .75	15.34 1.30	14.85 0.79	# 14.44 0.75	14.68 0.47	(p<0.00310)
Testes descent	.30 .48	25.10 0.31	25.00 0.00	25.37 0.61	25.13 0.35	
Vaginal opening	.11 .45	@^ 32.07 0.27	@^ 32.80 0.42	35.33 0.86	@^ 32.94 0.65	(p<0.00001)

#: Morphie..> the designated group .....mean
^:Eth-Mor-Hi.> the designated group .....mean
*:Eth-MorLo> the designated group .....mean
@: Ethanc ..> the designated group .....mean

Significant differences due to prenatal exposure were observed for incisor eruption ($F(4,112)=9.23$, $p<0.00001$). The Scheffe comparison of means indicated that incisor eruption was the slowest in the etOH-morphine (low)-exposed offspring.

Significant differences due to prenatal exposure were also evident for the development of primary coat ($F(4,112)=4.89$, $p<0.0007$). The Scheffe comparison of means revealed that the morphine-exposed offspring developed their fur coat sooner than the offspring exposed to either etOH, or etOH-morphine (high); and the offspring exposed to etOH-morphine (low) sooner than the offspring exposed to etOH-morphine (high).

Also significant were the differences due to prenatal exposure for eye opening ($F(4,112)=4.27$, $p<0.002$). According to the Scheffe comparison of means the offspring exposed to morphine opened their eyes later than the etOH-morphine (high)-exposed offspring.

Reflex Development

There were significant differences due to prenatal exposure for surface righting ($F(4,112)=8.63$, $p<0.00001$), and negative geotaxis ($F(4,112)=5.56$, $p<0.0033$). The Scheffe comparison of means indicated that the morphine-exposed offspring were the slowest to develop surface righting, and slower than the offspring exposed to etOH-morphine (low) to develop negative geotaxis.

Spontaneous Behavior

There were significant differences due to prenatal exposure for crawling ($F(4,112)=5.82$, $p<0.0002$). The Scheffe comparison of means revealed that the offspring exposed to morphine developed crawling later than the offspring exposed to either etOH-morphine (low), or etOH-morphine (high), or water. There were also significant differences due to prenatal exposure for walking ($F(4,112)=13.10$, $p<0.00001$). The Scheffe comparisons of the means revealed that the water-exposed offspring walked sooner than the offspring exposed to morphine, or etOH-morphine (high), or etOH-morphine (low).

Sexual Development

The degree of sexual development in the rat is generally assessed by the appearence of sperm in the male, and the onset of estrus in the female. However in a thorough investigation of the sexual development of the rat Satinder (1984) indicated that the degree of sexual maturation can also be measured by the descent of testes in the male, and the vaginal opening in the female.

There were no differences due to prenatal exposure for sexual development (i.e. descent of testes) of the male. However there were significant differences due to prenatal exposure for vaginal opening. The Scheffe comparison of the means revealed that the female offspring exposed to etOH, or to etOH-morphine (high), were slower to mature sexually than the remaining female offspring. These results confirmed existing reports by Esquifino et al (1986), and Barron, and Riley (1985).

Overall the morphine-exposed offspring were the slowest to develop, followed by the etOH-morphine (high) group, the etOH-, and the etOH-morphine (low)-exposed offspring.

Postnatal Fluid Consumption and Drug Preference

There were no significant differences due to prenatal exposure for water consumption on days 29, 30, and 34. Significant differences were observed for water consumption due to postnatal exposure on day 35 ($F(4,112)=5.96$, $p<0.0002$). The offspring exposed to etOH-morphine (high) consumed more water than the offspring exposed to etOH, morphine, and water, as verified by the Scheffe comparison of means (Figure 5 and Table 7).

The fluid consumption data for days 31, 32, and 33 were analyzed both in terms of the consumed fluid in ml, as well as the absolute drug content. Both analyses will be presented here starting with the discussion on the aspects of fluid consumption. All relevant measurements are listed in Tables 7, and 8.

There were significant differences for fluid consumption on day 31 due to prenatal exposure ($F(4,112)=4.40$, $p<0.0024$), and due to the order of presentation ($F(1,115)=14.34$, $p<0.0002$). The Scheffe comparison of means revealed that the etOH-morphine (high)-exposed offspring exhibited higher fluid consumption than the water-exposed animals. Regardless of prenatal exposure the group exposed to etOH on day 31, exhibited higher fluid consumption than the group exposed to morphine on the same day.

Figure 5. Mean Offspring Fluid Consumption vs Days

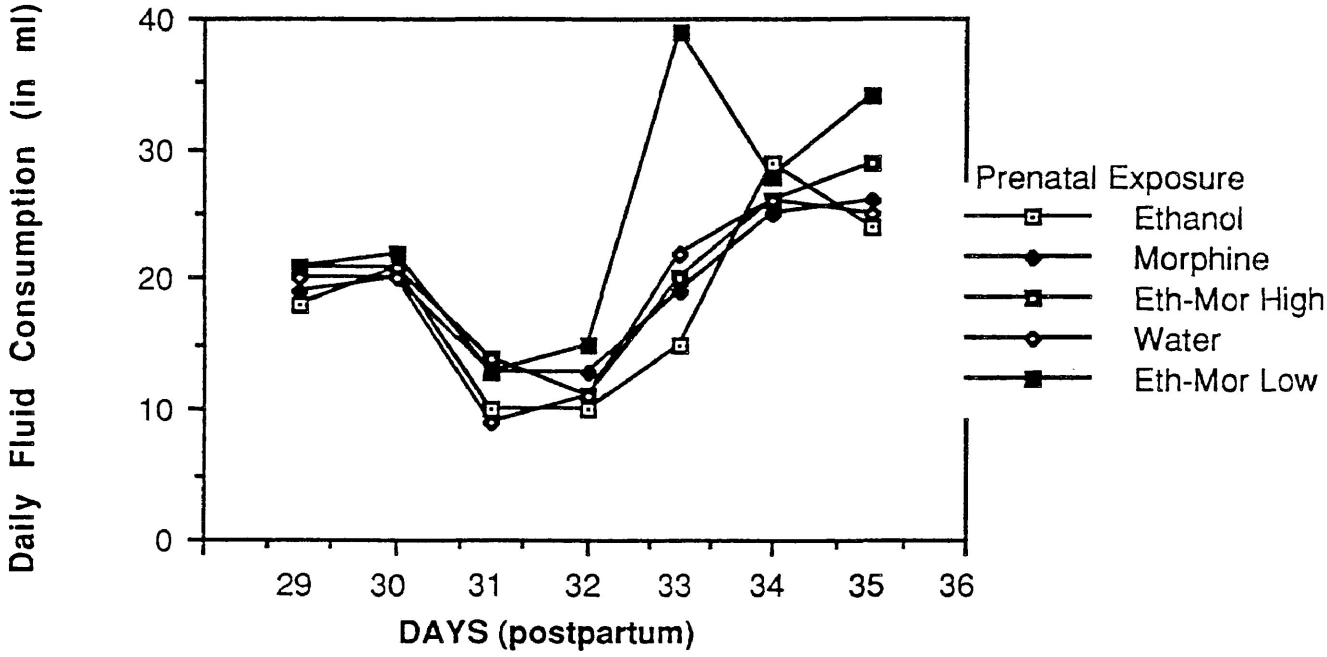


Table 7. Mean Fluid Consumption of Offspring (with SD) By Prenatal Exposure

Pren.Exposure	Ethanol	Morphine	Eth-Mor High	Eth-Mor Low	Water	Signific. Leve (1WayANOVA)
Mean (and SD)						
Day 29 postpartum	18.1 (3.28)	19.04 (5.52)	21.04 (5.76)	21.09 (5.27)	19.87 (2.47)	
Day 30 postpartum	20.84 (2.65)	20.26 (4.22)	21.50 (6.18)	21.76 (5.74)	19.87 (3.67)	
Day 31 postpartum	10.21 (4.07)	13.17 (5.58)	14.45 (5.36)	12.66 (5.92)	*8.93 (5.75)	(p<0.00240)
Day32 postpartum	10.10 (5.06)	13.04 (6.02)	11.27 (7.78)	14.76 (9.47)	11.09 (5.68)	
Day33 postpartum	^15.10 (3.81)	^19.04 (6.63)	^20.09 (7.67)	38.66 (17.40)	^22.53 (14.16)	(p<0.00001)
Day 34 postpartum	29.36 (7.50)	24.95 (8.23)	26.13 (7.74)	27.47 (9.63)	26.18 (6.94)	
Day 35 postpartum	^24.05 (5.11)	^26.60 (5.41)	29.72 (6.47)	33.85 (12.40)	^25.40 (6.20)	(p<0.00020)

*:Eth-Mor hi> the designated group mean
^:Eth-Mor Lo> the designated group mean

Table 8. Absolute Amount of Drug Consumed (mean with SD) By Prenatal Exposure

Pren.Exposure	Ethanol	Morphine	Eth-Mor High	Eth-Mor Low	Water	Signific. Leve (1WayANOVA)
Mean (and SD)						
Ethanol (ml) when forced	1.06 (0.46)	1.16 (0.49)	1.25 (0.59)	1.52 (0.69)	1.25 (0.32)	
Morphine (mg) when forced	4.76 (2.13)	7.26 (3.07)	6.61 (3.85)	6.07 (4.28)	*3.75 (3.38)	(p<0.0020)
Ethanol (ml) in choice	^1.08 (0.44)	^0.73 (.038)	^0.95 (0.50)	2.20 (1.35)	^1.37 (0.86)	(p<0.0001)
Morphine (mg) in choice	^2.13 (2.06)	6.26 (4.65)	5.50 (5.08)	8.31 (5.14)	4.42 (4.41)	(p<0.0005)
Preference for ethanol	0.72	@0.44	0.45	0.63	0.61	(p<0.0090)
Preference for morphine	*0.28	0.56	0.55	0.37	0.39	(p<0.0090)

@: Ethanol > the designated group mean
*: Morphine > the designated group mean
^: Eth-Mor Lo> the designated group mean

It has also been postulated that an effect on water drinking is possible after the administration of an opiate agonist (Spencer et al 1986). No studies however have been conducted on the effects of prenatal exposure on the postnatal drinking behavior. The study of postnatal water consumption (on days 29, 30, 34,and 35) revealed significant differences in water consumption due to prenatal exposure, on day 35 ($F(4,112)=5.46$, $p<0.0002$). Specifically the etOH-morphine (low) group exhibited significantly higher consumption than the groups exposed to etOH, morphine and water. Unfortunately the design of the study did not premit the monitoring of this effect to establish if it was a transient phenomenon or the expression of a permanent effect on development.

The study of absolute drug consumption revealed no differences due to prenatal exposure, order of presentation, or sex were observed for the levels of consumed etOH during the days of forced consumption (Table 8, Figure 6). There were differences due to prenatal exposure for morphine consumption ($F(4,112)=4.51$, $p<0.002$). According to the Scheffe comparison of the means the morphine-exposed offspring consumed more morphine than the water-exposed animals during the days of forced consumption (Figure 7).

Figure 6. Forced Ethanol Consumption vs Order

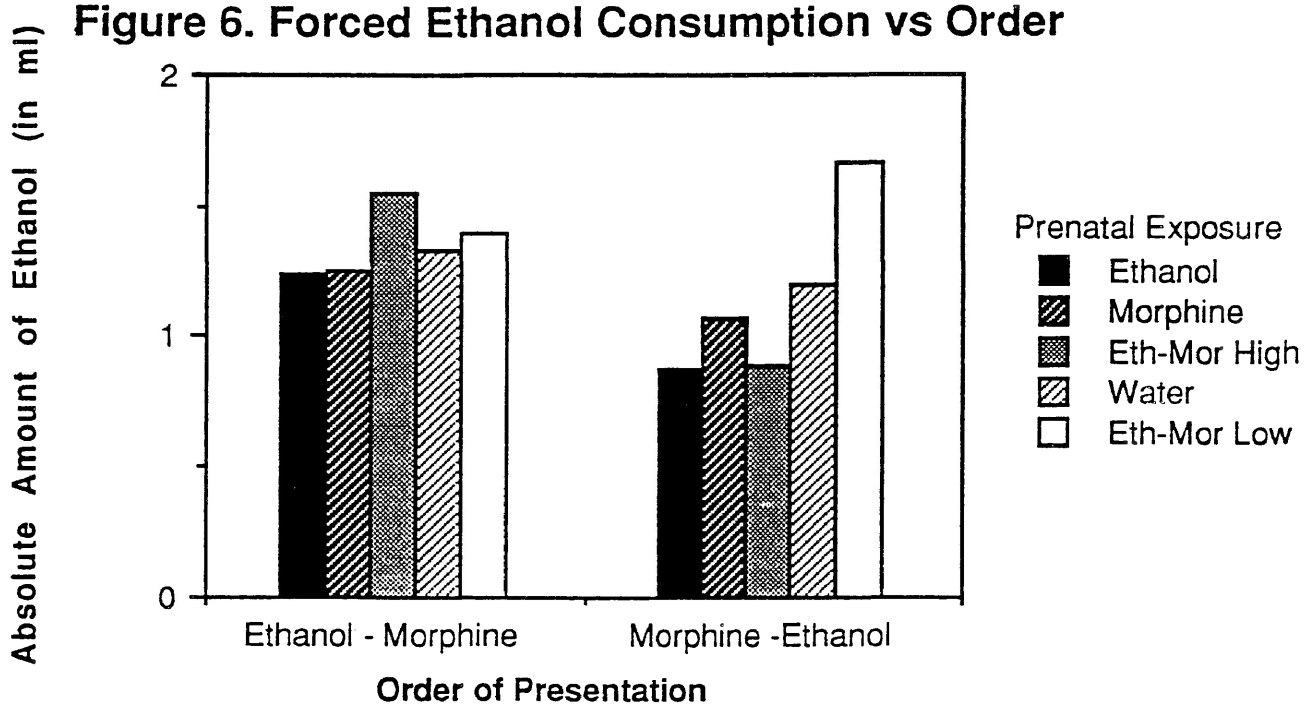


Figure 7. Forced Morphine Consumption vs Order

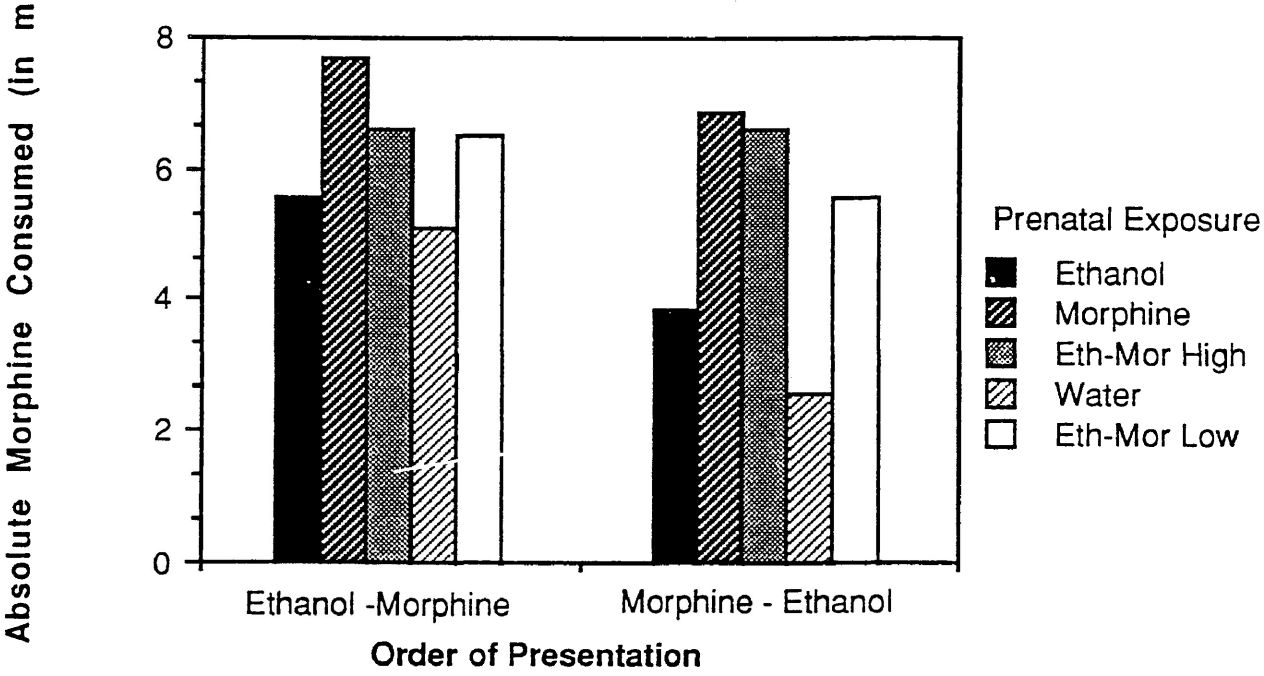


Figure 8. Choice Ethanol Consumption vs Order

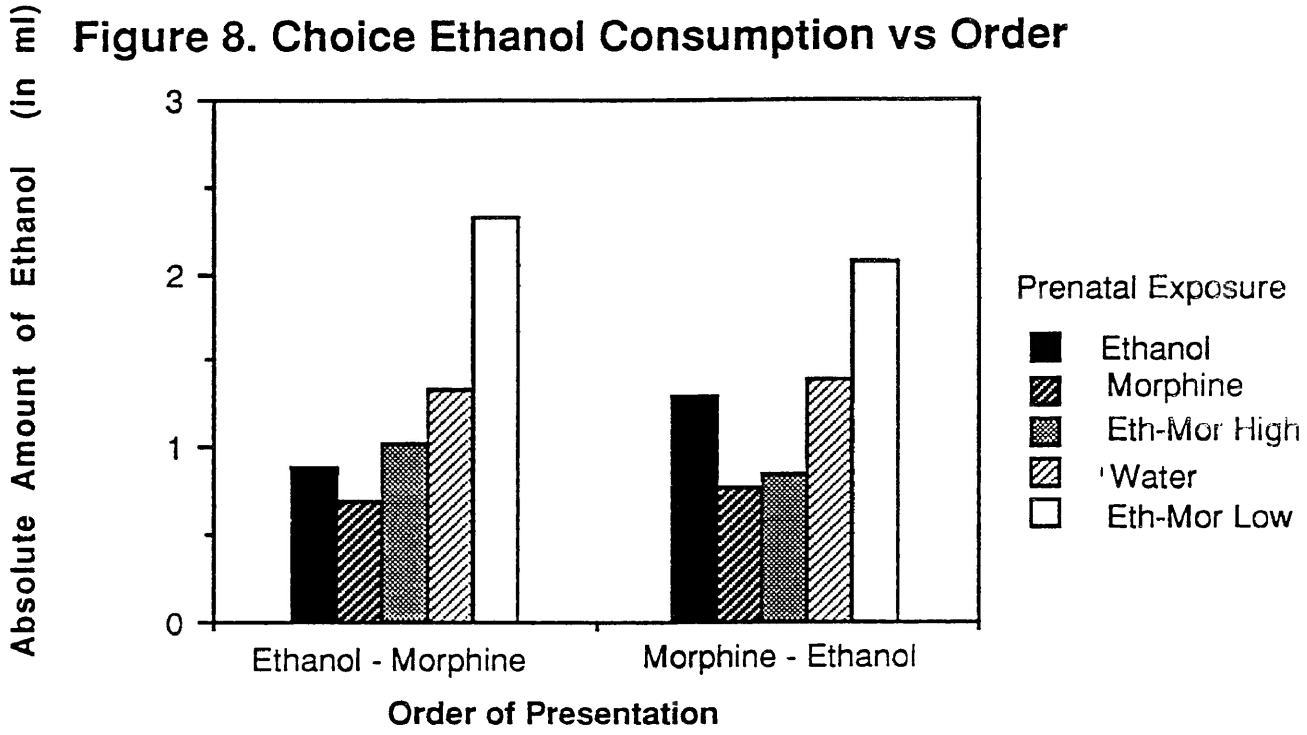
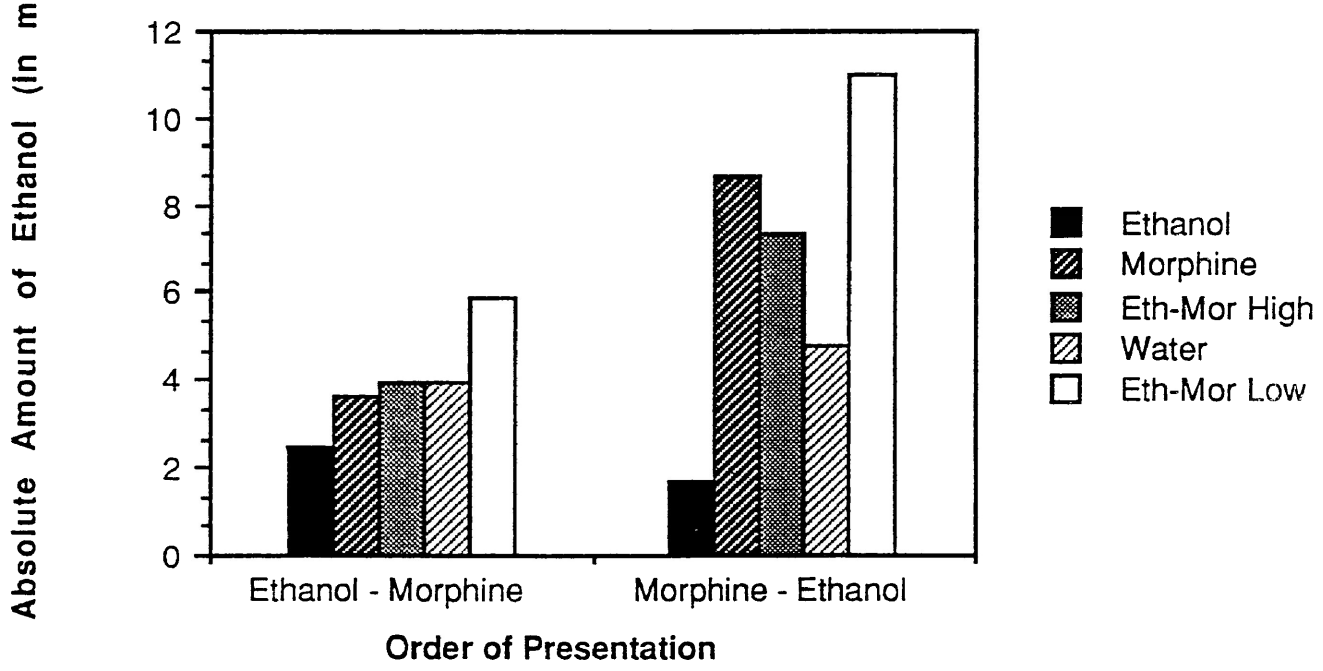


Figure 9. Choice Morphine Consumption vs Order



There were significant differences due to prenatal exposure for etOH consumption on the day of choice($F(4,112)=10.88$, $p<0.00001$). The Scheffe comparison of the means revealed that the etOH-morphine (low)-exposed offspring had the highest consumption of etOH (Figure 8). There were also significant differences due to prenatal exposure ($F(4,112)=5.37$, $p<0.0005$), and order ($F(1,115)=9.47$, $p<0.0026$), on the day of choice. The Scheffe comparison of the means indicated that the etOH-morphine (low)-exposed offspring consumed more morphine than the etOH-exposed group. Also the group that experienced forced morphine consumption first exhibited the highest consumption of morphine (Figure 9).

In order to estimate any differences in the preference for etOH, or morphine, on the day of choice, a preference rate was calculated as the ratio of the partial (etOH, or morphine) volume over the total (etOH and morphine) volume consumed. These ratios are listed in Table 8. There were significant differences due to prenatal exposure for both the etOH ($F(4,112(=3.87$, $p<0.005$), and the morphine ($F(4,112)=3.87$, $p<0.005$) preference rates.

The Scheffe comparisons of these means revealed two reverse relationships. The etOH-exposed group exhibited the highest preference for etOH, but the ratio compared significantly only with the preference of the morphine-exposed group. On the other

hand the morphine-exposed group exhibited the highest preference for morphine, but this ratio was only significant when compared with the preference of the etOH-exposed group.

The above data revealed an influence of the prenatal exposure on the postnatal consumption of etOH or morphine when the order of presentation was considered. Both the etOH- and the morphine-exposed groups showed a relative preference towards the agent to which they were prenatally exposed. When the preference ratios were further evaluated with the sign test, it was revealed that the offspring exposed to etOH exhibited the highest preference for etOH on the day of choice (χ^2 (1)=18.48, $p<0.01$). The group exposed to etOH-morphine (low) exhibited a similar preference for etOH (χ^2 (1)=6.25, $p<0.015$), while the group exposed to water exhibited the least preference for etOH ($\chi^2(1)=4.4$, $p<0.025$). The prenatal exposure to the combination of the two agents seemed to be dose-related. While the low dose of etOH-morphine resulted in increased etOH preference the high dose had an effect similar with the prenatal exposure to morphine.

Overall the offspring seem to exhibit a slight preference towards the etOH over the morphine solutions. However this "natural" tendency was reversed due to prenatal exposure. The implications of this reversal are discussed in a later section.

Postnatal Food Consumption Patterns

The data on food consumption are summarized in Figure 10. There were significant differences due to prenatal exposure for food consumption on day 29, and the group exposed to etOH-morphine (high) consumed more food than the etOH-exposed offspring, according to the Scheffe comparison of means. On day 30 the differences in food consumption due to prenatal exposure were also significant ($F(4,112)=4.41$, $p<0.0024$), and the offspring exposed to water consumed more food than the animals in the etOH-morphine (high) group as indicated by the Scheffe comparison of means. Significant differences due to prenatal exposure for food consumption were again evident on day 32 ($F(4,112)=3.63$, $p<0.0079$), but no specific group differences were revealed by the Scheffe comparison of means. Differences due to prenatal exposure for food consumption were also evident on day 34 ($F(4,112)=5.35$, $p<0.0006$). The Scheffe comparison of means indicated that during that time the offspring exposed to etOH, consumed more food than the offspring exposed to etOH-morphine (low), or etOH-morphine (high).

Figure 10. Postnatal Food Consumption vs Days

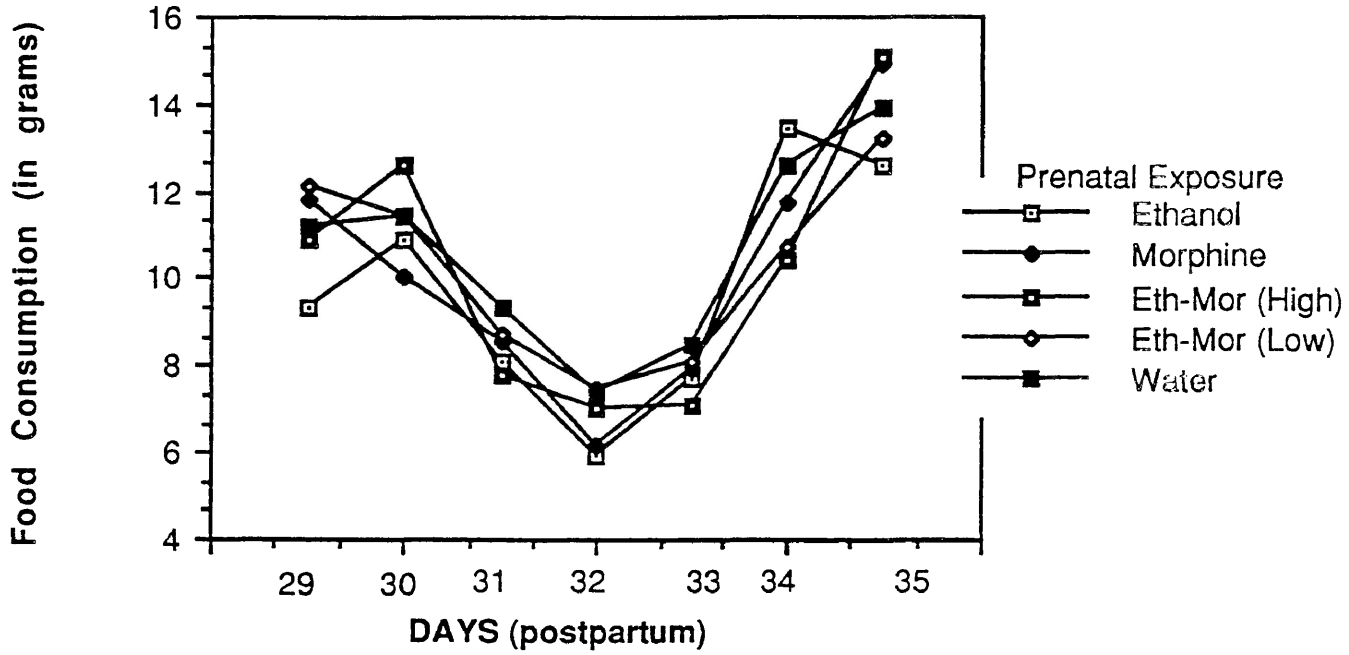
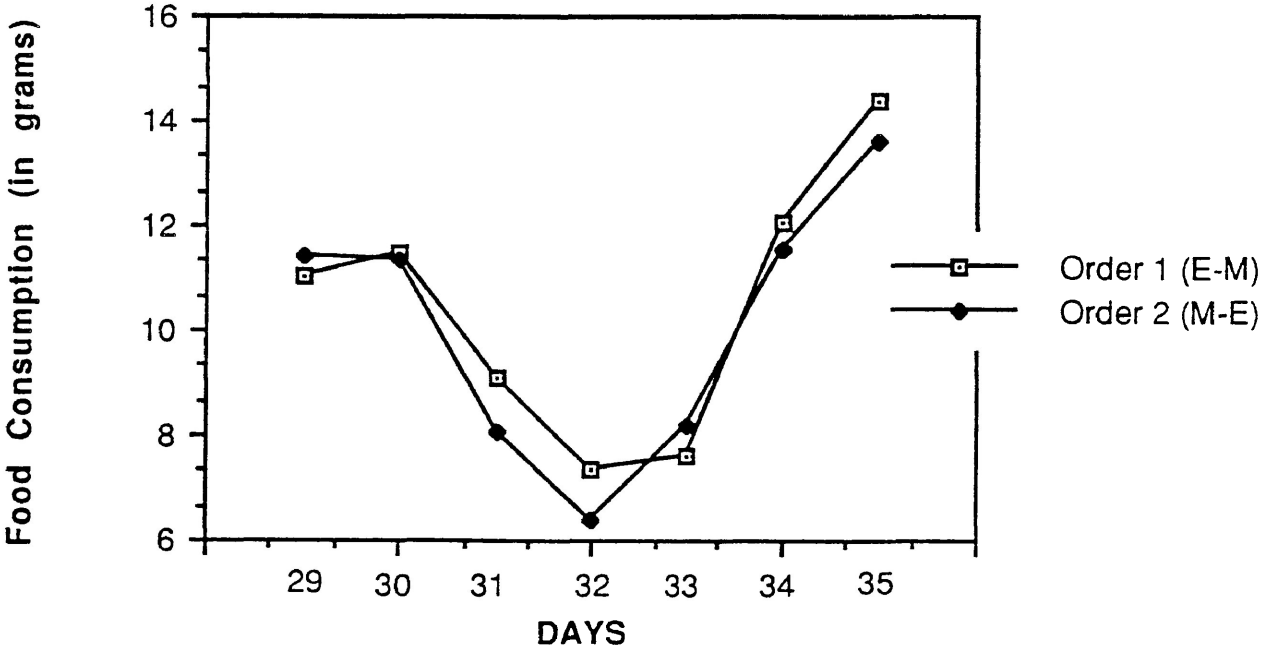


Figure 11. Postnatal Food Consumption vs Days by Order



When the order of drug presentation during days 31, and 32 was considered (i.e. etOH-morphine, or morphine-etOH) there were significant differences for food consumption on both days 31 ($F(1,115)=8.06$, $p<0.0053$), and 32 ($F(1,115)=8.63$, $p<0.0040$) (see Figure 11).

There are no reports in the literature relating prenatal exposure to etOH, or opiates with changes in postnatal eating behavior. The differences in postnatal food consumption observed in this study did not reveal any systematic changes. However when the order of drug presentation on days 31, and 32 was considered, consistent changes were observed. The food consumption was reduced for both groups, but more for the group exposed to morphine first. The recovery of food consumption was faster in the same group and started on day 33. The animals exposed to the etOH first, recovered their food consumption on day 34 (see Figure 11). It has been stated before that the etOH reduces the food motivation of the organism (Sterling and Smith, 1977; Fernandez et al, 1983; Testar et al, 1988). The effect of morphine on eating however has not been conclusively determined (Olson et al, 1986). It appears though that in the present study the two agents, presented in sequence exhibited a synergistic effect in the reduction of the motivation for food.

Reactivity Testing

The traditional four-day testing in the open-field was replaced by a 1 min observation period in the circular runway (the avoidance response apparatus). There were no differences due to prenatal exposure, or sex for latency to begin exploration, number of sections crossed, time spent exploring, and defecation. There were however significant differences due to prenatal exposure for number of returns to the starting point ($F(4,112)=3.72$, $p<0.007$). The Scheffe comparison of means revealed that the offspring exposed to etOH-morphine (high) returned to the starting point more often than the water-exposed offspring (Table 9).

Unconditioned Escape Response

There were significant differences due to prenatal exposure for the UER ($F(4,112)=3.08$, $p<0.01$). Overall the following relationship was observed for the average UER's of the various groups: etOH > water > etOH-morphine (high) > morphine > etOH-morphine (low). However none of these trends were revealed significant by the Scheffe procedure (Table 10).

Table 9. Reactivity Measures (mean with SD) By Prenatal Exposure

Pren.Exposure	Ethanol	Morphine	Eth-Mor (high)	Eth-mor (low)	Water
Latency (sec.)	21.5 11.2	24.5 15.3	20.2 10.5	26.5 13.9	24.9 15.1
Time Out (sec.)	34.3 9.3	28.3 13.6	29.9 10.0	28.3 13.4	31.0 14.0
Sections Crossed	4.9 1.4	3.9 2.2	4.4 2.0	3.5 2.2	4.2 1.9
Returns to Start point	0.4 0.6	0.6 0.6	0.8 0.5	0.4 0.6	* 0.2 0.4
Defecation	0.05 0.2	0.2 0.6	0.3 0.9	0.0 0.0	0.0 0.0

*: Eth-Mor>(high) Water

Table 10. Mean Unconditioned Escape Response (UER) in mA (with SD) By Prenatal Exposure

Pren Exposure	Ethanol	Morphine	Eth-Mor High	Eth-Mor Low	Water	Signific Level (1WayANOVA)
UER (mean with SD)						
Females	0.35 (0.05)	0.28 (0.05)	0.27 (0.06)	0.29 (0.05)	0.33 (0.07)	
Males	0.33 (0.04)	0.30 (0.06)	0.31 (0.08)	0.28 (0.04)	0.30 (0.03)	
Total	0.34 (0.05)	0.29 (0.06)	0.30 (0.07)	0.28 (0.04)	0.31 (0.05)	(p<0.001)

Table 11. Mean Avoidance Responses By Prenatal Exposure

Pren.Exposure	Either Way	One Way	Two Way
Ethanol	3.3	2.5	0.8
Morphine	3.3	1.9	1.3
Eth-Mor (high)	5.0	3.6	1.3
Eth-Mor (low)	4.1	2.3	1.7
Water	3.4	2.0	1.4

Table 12. Significance Levels for One-way ANOVA (simple effects) Between Groups of different prenatal exposures for the One-way avoidance response.

Prenatal Exposure:

	etOH	Morphine	etOH-mor (high)	etOH-mor (low)	Water
etOH		NS	0.001	NS	NS
Morphine			0.0029	NS	NS
etOH-mor (high)				0.0122	0.0017
etOH-mor (low)					NS
Water					

NOTE
NS = Not Significant

Conditioned Avoidance Response

There were no differences due to prenatal exposure, or sex for the either-way or the two-way avoidance response. The results to be reported in this section refer to the one-way response alone. The data have been summarized in Table 11.

There were significant differences due to prenatal exposure for the one-way avoidance response on day 1 ($F(4,112)=5.10$, $p<0.0008$), and day 2 ($F(4,112)=4.85$ $p<0.0012$) of the avoidance training. There were no significant differences observed on days 3, and 4. The Scheffe comparisons of the means revealed that on day 1 the offspring exposed to etOH-morphine (high) performed more one-way avoidance responses than the offspring exposed to etOH, or water, or etOH-morphine (low).

On day 2 the offspring exposed to etOH-morphine (high) performed more one-way avoidance responses than the groups exposed to water, and morphine. There were significant differences due to prenatal exposure for the total one- way avoidance responses ($F(4,112)=4.02$, $p<0.0043$). The Scheffe comparison of means indicated that the group exposed to etOH-morphine (high) performed more one-way responses than the groups exposed to either morphine, or water.

The effects of prenatal exposure for the avoidance response were further analyzed through ANOVA comparisons of simple effects. With the exception of the etOH-morphine (low)-exposed offspring all other animals exhibited significantly lower one-way avoidance rates than the offspring exposed to etOH-morphine (high). These results are summarized on Table 12.

Lochry et al (1986) had suggested the use of tasks of low to moderate difficulty in order to avoid floor or ceiling effects due to either the effects of the task or the effects of the exposure. In the present study the differences in learning due to prenatal exposure disappeared for the task of two-way avoidance. It was earlier discussed however (see page 30), that the two-way avoidance response is more complex than the one-way avoidance response. The lack of differences for the two-way response in this study can to a certain degree be attributed to the difficulty of the task.

Hot-Plate Response

No differences due to prenatal exposure, body weight, or sex were found for the latency to respond in the hot plate. The finding contradicts existing reports of differences in hot-plate nociception due to prenatal exposure (Zagon and Mclaughlin, 1982).

The results of this study suggest that the exposure to prenatal etOH, morphine, and etOH-morphine (high) was behaviorally and developmentally toxic. The dose differences observed between etOH-morphine (high) and etOH-morphine (low), confirmed the hypothesis that the intensity of the prenatal exposure determines the effect of the teratogen (Ernhart et al, 1987). Furthermore the combination of the two agents in both doses seemed to have a lesser impact on the postnatal development and mortality of the offspring, than the exposure to either etOH or morphine alone. The discussion of these results and their implications will be presented in the following section.

DISCUSSION

Hutchings (1985) has discussed the possibility of an indirect effect of the prenatal treatment on the offspring. Since many maternal behaviors are sensitive to chemical exposure it is possible that the development will be affected depending on the effect the prenatal agent had on the mother. To exclude these effects from the analyses one needs to employ cross-fostering, or whenever that is impossible, one can introduce a systematic observation of the maternal behavior. Such an examination was included in this study. The observations did not reveal any differences in the maternal behaviors of the dams exposed to the different experimental conditions.

One of the most commonly cited problems in the study of drug-induced behavioral teratogenesis is the administration of the drug. In the case of etOH and morphine various methods have been devised to ensure uniform consumption among animals. Examples of the methods include oral gavage, liquid diets, and injections. Some of the methods are considered very intrusive and their disadvantages have often been discussed (Abel, 1980). The present study was characterized by a uniform fluid consumption among prenatal treatments. Only the dams exposed to water exhibited an overall higher fluid consumption indicating that the solutions of etOH and/or morphine were equally

aversive. Similarly the assumption of isocaloric content among etOH and/or morphine solutions was correct. As shown before there were no differences in the prenatal consumption of the etOH and/or morphine solutions. We may safely assume that there was an equal contribution of dietary calories from either the etOH, and/or the sugar, since the reduction of the food drive was fairly equal among the groups exposed to any of the drug regimens.

One may assume that the reduced food and water consumption exhibited by the dams exposed to etOH, morphine and etOH-morphine (high and low) may have had a detrimental effect of its own on the postnatal development. However no common patterns of physical or behavioral teratogenesis were identified among conditions thereby suggesting, that the observed results were due to a combination of factors unique to each condition and not to the reduction of food drive that was common in the etOH and/or morphine exposure. The findings of this study verified previous reports (Fernandez et al, 1986, and Testar et al, 1988), that the reduced food consumption alone, is not sufficient to account for the effects of prenatal etOH exposure on the offspring.

The most clear demonstration of the prenatal influence of the exposure on postnatal behaviors was observed during the preference testing of the offspring. Overall the etOH solutions

were more palatable than the morphine solutions. Slight preference rates for morphine were exhibited only by the morphine- and the etOH-morphine (high)-exposed groups. The prenatal exposure did not influence the preference for etOH, although it appeared to slightly increase the palatability of the etOH solutions. For instance the etOH-, and the etOH-morphine (low)-exposed offspring showed higher preference for etOH than the water-exposed animals. The prenatal exposure to morphine however seemed to have a more dramatic effect on postnatal preference. Namely the "natural" preference for etOH over morphine solutions (exhibited by the water-exposed animals) was reversed as a result of prenatal exposure. The results suggest that following certain types of prenatal exposure the offspring might be at a risk of either inducing a higher consumption level of an addictive substance or consuming an addictive substance which can be "naturally" aversive. Relevant observations had indicated that animals which avoided solutions of morphine on a first encounter, continued avoiding the solution even after an extensive period of fluid (or water) deprivation.

The clinical implications of the above suggestion are obvious and further discussion and study of the phenomenon is required to delineate all the involved factors. For instance Anokhina and Gamaleya (1986), found that the blood serum of alcoholics contained increased numbers of antibodies to

morphine. One may then assume that the same occurrence is also true for the blood serum of an morphine-using mother. The increased dependence of the offspring on the mother for its food supply will also influence the development of its own immune system. It is therefore possible that the developing offspring will develop antibodies to substances (i.e. morphine) that are present in the blood and subsequently the milk of the mother.

There was no provision in this this study to investigate the possibility of the above assumption. The need therefore remains to correlate the effects of prenatal administration on specific physiological mechanisms. The most appropriate recommendation for future investigations of this type appears to be the inclusion of an immunological measure.

The study of learning behavior revealed only a mild effect of prenatal exposure on the development of the avoidance response. Bond (1981) in reviewing relevant evidence concluded that the effects of prenatal ethanol on learning mainly depend on the nature of the task. Other investigators have reached similar conclusions (Pierce, and West, 1986). As Lochry (1986) has shown the effects of prenatal etOH on learning for the most part depend on the dose of exposure or the degree of difficulty of the task.

In the literature there are no reports of any direct

effects of prenatal morphine exposure on learning. Instead the effects of morphine are measured indirectly by assessing the tolerance of the exposed animal to the learning-disruptive effects of morphine administration (Wagner et al,1986).

The present study showed differences in learning, due to prenatal exposure, only for the one-way avoidance response, and only for the etOH-morphine (high) exposed offspring. The results of the present study imply that the combination of the two agents has a significant synergistic effect on learning, while the effects of individual agents on learning are not discernable.

It is therefore possible that the effects of prenatal exposure on learning might be apparent only after the postnatal repetition of the prenatal experience. It might therefore be necessary to include the study of the tolerance to the effects of the agent on learning. This method has been efficiently used in the past (Nelson et al,1986 a, and b).

Similar explanations also apply for the lack of differences observed for hot-plate nociception, since Zagon and Mclaughlin (1982) have reported differences in nociception following postnatal repetition of the prenatal experience. Following the above discussion it is recommended that the paradigm of this study is modified to acommodate the above considerations.

Future designs should include the study of learning and nociception following the administration of the prenatal agent. However one should note that the continuous presentation of the prenatal agent will alter the nature of the results. It is evident that the continuous administration of the prenatal agent will result in a postnatal pharmacological effect, which might not be related to the prenatal administration. We therefore recommend the use of designs that permit the fewest possible administrations of the prenatal agent and still permit the adequate study of the underlying phenomena. For instance one feasible design would include the study of learning, or nociception, following postnatal administration of the prenatal agent, but in different repetitions of the experiment. Similar developments will improve both the external and the internal validity of the resulting designs, that by necessity remain complex.

The reactivity measures for instance revealed no differences due to prenatal exposure. It is possible that the paradigm of activity testing, employed in this study did not allow sufficient time for the proper sampling of differences in activity. It is very likely that the use of another testing paradigm for reactivity (such as open-field) would be more sensitive to identify activity differences due to prenatal exposure. There has been at least one report that prenatal exposure to morphine results in higher open-field activity

(Davis, and Lin, 1972). In addition prenatal exposure to etOH has also been found to produce hyperactivity depending on the dose of etOH available to the offspring (compare Middaugh et al, 1988; Randal et al, 1986).

The use of a test for reactivity is also recommended, despite the lack of differences due to prenatal exposure. However the used design appears inadequate to measure any differences. A more appropriate design has been used by Davis and Lin (1972).

Despite the need for future improvements the present study has produced interesting evidence about the relationship of etOH and morphine. The results confirmed that there is a functional relationship between the two agents. In past research though, etOH and morphine have been considered to be related in a synergistic fashion (Ng and Amit, 1985; Rae, 1986; Nelson et al, 1986). The concurrent administration of the two agents however changed that relationship into an antagonistic one. As a result no additive effects were observed following exposure to etOH-morphine. Instead the impact of the insult was lessened indicating a "protective" influence of the concurrent administration, from the effects of the single administration. The most dramatic demonstration of this trend was evident in the offspring of the etOH-morphine (low) exposure. These animals exhibited only mild behavioral deficits and normal development,

compared to offspring exposed to either etOH or morphine alone.

Based on the findings of this study there is further need for investigation and documentation of the interaction effects observed when the exposure of etOH and morphine were combined. Future research should focus on the development of a paradigm sensitive enough to identify the differences in learning, and activity and exclusive of the methodological flaws that were presented here. There is also need to clearly demonstrate the dose-related differences. Most importantly however there is the need to demonstrate the actual physiological location at which these interactions take place. The suggestion for future research would be to correlate the preference, learning, and activity data with the relative changes in the immunoreactivity of the organisms following prenatal exposure.

REFERENCES

Abel, E.L. (1980) Procedural Considerations in Evaluating Prenatal Effects of Alcohol in Animals. Neurobehavioral Toxicology 2(3) :167-174.

Abel, E.L. (1984) Fetal Alcohol and Fetal Alcohol Effects. Plenum Press, New York.

Abel, E.L., and B.A. Dintcheff (1978) Effects of Prenatal Alcohol Exposure on Growth and Development in Rats. The Journal of Pharmacology and Experimental Therapeutics 207 :916-921.

Anokhina, I.P., N.B. Gamaleya, and T.P. Nebarakova (1988) Antibodies to Morphine and to Neurotransmitters in the Blood Serum of Chronic Alcoholics. Bulletin of Experimental Biology and Medicine 104 : 460-462.

Balakleevskii A.I., I.V. Maslova, S.V. Petrenko, and P.M. Surikov (1986) Enkephalin and Cyclic Nucleotide Levels in Brain Structures of Rats at Different Stages of Alcohol Dependence. Bulletin of Experimental Biology and Medicine 101 :288-291.

Barron, S., and E.P. Riley (1985) Pup-Induced maternal Behavior in Adult and Juvenile Rats Exposed to Alcohol Prenatally. Alcoholism: Clinical and Experimental Research 9 :360-365.

Beaman, C.M., G.A. Hunter, L.L. Dunn, L.D. Reid (1984) Opioids, Benzodiazepines and Intake of Ethanol. Alcohol 1 :39-42.

Bignami, D.A., (1965) Selection for High Rates and Low Rates of Avoidance Conditioning in the Rat. Animal Behaviour 13 :221-227.

Bond, N. (1981) Effects of Prenatal Ethanol Exposure on Avoidance Conditioning in High- and Low- Avoidance Rat Strains. Psychopharmacology 74 :177-181.

Broadhurst, P.L. (1975) The Maudsley Reactive and Nonreactive Strains of Rats : a Survey Behavioral Genetics 5 :299-319.

Burns, E.M., T.W. Kruckeberg, M.F. Kanak, and H. Stilber (1986) Ethanol Exposure During Brain Ontogeny: Some Long-Term Effects Neurobehavioral Toxicology and Teratology 8 :383-389.

Cardinale, G.J., J. Donnerer, A.D. Finck, J.D. Kantrowitz, K. Oka, and S. Spector (1987) Morphine and Codeine are Components of Human Cerebrospinal Fluid. Life Sciences 40 :301-306.

Castellano, C., and M. Amassari-Teule (1984) Prenatal Exposure to Morphine in Mice: Enhanced Responsiveness to morphine and stress. Pharmacology Biochemistry and Behavior 21 :103-108.

Chassnoff, I.J., W.J. Burns (1984) The Moro Reaction: A Scoring System for Neonatal Narcotic Withdrawal. Dev Med and Clin Neurology 26 :484-489.

Davis, W.M., C.H. Lin (1972) Prenatal Morphine Effects on Survival and Behavior of Rat Offspring. Research Communications In Chemical Pathology and Pharmacology 3(2) :205-214.

Davis, V.E., and, M.J. Walsh (1970) Alcohol, Amines and Alkaloids: a possible Basis for alcohol Addiction. Science 167 :1005-1007.

Ernhart C.B., R.J. Sokol, S. Martier, P. Moron, D. Nadler, J.W. Ager, and A. Wolf (1987) Alcohol Teratogenicity in the Human: a Detailed Assessment of Specificity, Critical Period and Threshold. American Journal of Obstertrics ang Gynecology 156 :221-235.

Esquifino, A.I., R Sanchis, and C. Guerri (1986) Effect of Prenatal Alcohol Exposure on Sexual Maturation of Female Rat Offspring. Neuroendocrinology 44 :483-487.

Fernandez K., W.F. Caul, M. Haenlein, and C.V. Vorhees (1983) Effects of Prenatal Alcohol on Homing Behavior, Maternal Responding and Open-Field Activity in Rats. Neurobehavioral Toxicology and Teratology 5 :351-356.

Hamel, E., and A. Beaudet (1987) Opioid Receptors in Rat Neostratum: Radioautographic Distribution at the Electron Microscopic Level

Harpel, H.S., and R.F. Gautieri (1968) Morphine-Induced Fetal Malformations I : Exencephaly and Axial Skeletal Fusions. Journal of Pharmaceutical Sciences 57 1590-1597.

Hilakivi, L. (1986) Effects of Prenatal Alcohol Exposure on Neonatal Sleep-wake Behavior and Adult Alcohol Consumption in Rats. Acta Pharmacologica et Toxicologica 59 :36-42.

Hovious, J.R., and M.A Peters (1985) Opiate Self-Administration in Adult Offspring of Methadone-Treated Female Rats. Pharmacology Biochemistry and Behavior 22 :949-953.

Hutchings, D.E. (1982) Methadone and Heroin During Pregnancy: A review of Behavioral Effects in Human and Animal Offspring. Neurobehavioral Toxicology and Teratology 4 :429-434.

Hutchings, D.E. (1985) Issues of Methodology and Interpretation in Clinical and Animal Behavioral Teratology Studies. Neurobehavioral Toxicology and Teratology 7 :639-642.

Hwang, B.H. (1986) Increased Synaptic Contacts of Catecholaminergic Boutons in The Cerebral Cortex and Paraventricular Hypothalamic Nucleus of Rats After Prenatal and Perinatal Ethanol Exposure. Experimental Neurology 92 :762-768.

Jensh, R.P. (1981) Behavioral Testing Procedures: A Review. In: The Biochemical Basis of Chemical Teratogenesis. By: M.R. Juchan (ed) :171-199. Elsevier/North Holland, New York.

Kirby, M.L., S.E. DeRosset, and S.G. Holtzman (1982) Enhanced Analgesic Response to Morphine in Adult Rats Exposed to Morphine Prenatally. Pharmacology Biochemistry and Behavior 17 :1161-1164.

Kreek, M.J. (1983) Opioid Interactions with Alcohol. Proc. MSC, NCA, Houston, Texas, April 1983.

Lochry, E.A., A.M. Hoberman, M.S. Christian (1986). Detection of Prenatal Effects as a Function of Learning Criteria. Neurobehavioral Toxicology and Teratology 7 :697-701.

Maizelis, M.Y., and A.L. Zabludovskii (1986) Enkephalin Level in Brain Regions of Rats Prenatally Exposed to Ethanol Bulletin of Experimental Biology and Medicine 101 :315-316.

McLean, S., R.B. Bothman, A.E. Jacobson, K.C. Rice, and M. Herkenham (1987) Distribution of Opiate Receptor Subtypes and Enkephalin and Dynorphin Immunoreactivity in the Hippocampus of Squirrel, Guinea Pig, Rat, and Hamster. The Journal of Comparative Neurology 255 :497-510.

Meyer, L.S.,and E.P. Riley (1986) Social Play in Juvenile Rats Prenatally Exposed to Alcohol. Teratology 34 :1-7.

Middaugh, L.D., C.L. Randall, and J.P. Favara (1988) Prenatal Ethanol Exposure in C57 Mice: Effects on Pregnancy and Offspring Development. Neurotoxicology and Teratology 10 :175-180.

Millan, M.J. (1986) Multiple Opioid Systems and Pain. Pain 27 :303-347.

Nathaniel, E.J.H., D.R. Nathaniel, S.A. Mohamed, L. Nahnybida, and L. Nathaniel Growth Patterns of Rat Body , Brain, and Cerebellum in Fetal Alcohol Syndrome. Experimental Neurology 93 :610-620.

Neil A., V. Kayser, G. Gacel, J.M. Besson, and G. Guilbaud (1986) Opioid Receptor Types and Antinociceptive Activity in Chronic Inflammation: Both kappa- and mu-Opiate Agonistic Effects are Enhanced in Arthritic Rats. European Journal of Pharmacology 130 :203-208.

Nelson, L.R., A.N. Taylor, J.W. Lewis, B.J. Branch, and J.C. Liebeskinnd (1986) Morphine Analgesia is Potentiated in Adult Rats Prenatally Exposed to Ethanol. Brain Research 372 :234-240

Nelson, L.R., A.N. Taylor, J.W. Lewis, R.E. Russell, E. Rdei, and B.J. Branch (1986) Pituitary-Adrenal Responses to Morphine and Footshock Stress are Enhanced Following Prenatal Alcohol Exposure. Alcoholism: Clinical and Experimental Research 10(4) :397-402.

Nelson, L.R., J.W. Lewis, N. Kokka, B.J. Branch, and A.N. Taylor (1986) Prenatal Exposure to Ethanol Potentiates Morphine-Induced Hypothermia in Adult Rats. Neurobehavioral Toxicology and Teratology 8 :469-474

Ng Cheong Ton,J.M. and Z. Amit (1985) Stereospecific Interaction Between Alcohol and Opiates Mediated by the mu High-Affinity Binding Receptor. Alcohol 2 :333-337.

Norton, S. (1978) Is Behavior or Morphology a More Sensitive Indicator of Central Nervous System Toxicity? Environmental Health Perspectives 26 :21-27.

Olson G.A., Olson R.D., and A.J. Kastin (1986) Endogenous Opiates: 1985. Peptides 7 :907-933.

Pierce, D.R., and J.R. West (1986) Blood Alcohol Concentration: a Critical Factor for Producing Fetal Alcohol Effects. Alcohol 3 :269-272.

Rae, H.G. Retrospective Review of Ethanol Concentration Measurements in Patients with Ethanol Overdose Treated with Naloxone. Alcohol 40(5) :960-964.

Randall, C.L., H.C. Becker, and L.D. Middaugh (1986) Effects of Prenatal Ethanol Exposure on Activity and Shuttle Avoidance Behavior in Adult C57 mice. Alcohol and Drug Research 6 :351-360, 1986.

Reid L.D., and G.A. Hunter (1984) Morphine and Naloxone Modulate Intake of Ethanol. Alcohol 1 :33-37.

Robinson, R.O. (1977) Fetal Alcohol Syndrome. Developmental Medicine and Child Neurology 19 :538-540.

Rockwood, G.A., and E.P. Riley (1986) Suckling Deficits in Rat Pups Exposed to Alcohol in Utero. Teratology 33 :145-151.

Ronnback L., and E. Hansson (1986) Stimulation of Brain-stem Protein Synthesis by Morphine. Biochemical Pharmacology 35(21) :3685-3692.

Satinder, K.P. (1976) Sensory Responsiveness and Avoidance Learning. Journal of Comparative and Physiological Psychology 90 :946-957

Satinder K.P. (1980) Genetically Heterogeneous and Selected Lines of Rats: Behavioral and Reproductive Comparison Behavior Genetics 10 :191-200.

Satinder, K.P. (1982) Alcohol-Morphine Interaction: Oral Intake In Genetically Selected Maudsley Rats. Pharmacology Biochemistry and Behavior 16 :707-711.

Satinder, K.P. (1984) Biological Sexual Maturation in Rats: Interaction Among Genetic Line, Sex, Age, and Body Weight. Animal Reproduction Science 6 :311-322.

Sedlacek, J. (1986) Opioid Elements in Development of the Spontaneous Motility of Chick Embryos. Physiologia Bohemoslovaca 35 :427-436

Siegel, S. (1986) Alcohol and Opiate Dependence. Re-evaluation of the Victorian Perspective. In: Research Advances in Alcohol and Drug Problems Vol 9. By: H.D. Capell, F.B. Glaser, Y. Israel, H. Kalant W. Schmidt, E.M. Sellers, and R.C. Smart (eds). Plenum Publishing Co.

Sobrian, S.K. (1977) Prenatal Morphine Administration Alters Behavioral Development in the Rat. Pharmacology Biochemistry and Behavior 7 :285-288.

Spencer R.L., D. Deupree, S. Hsiao, H.I. Mosberg, V. Hruby, T.F. Burks , and F. Porreca (1986) Centrally-Administered Opioid Selective Agonists Inhibit Drinking in the Rat. Pharmacology Biochemistry and Behavior 25 :75-82.

Sterling, K.C. and D.W. Smith (1977) The Fetal Alcohol Syndrome. The New England Journal of Medicine 298(19) :1063-1067.

Suter, K.E., and H. Schon Testing Strategies in Behavioral Teratology: 1. Testing Battery Approach. Neurobehavioral Toxicology and Teratology 8 :561-566.

Szeto, H.H., Y.S. Zhu, J. Amione, and S. Clare (1988) Prenatal morphine Exposure and Sleep-Wake Disturbances in the Fetus. Sleep 11(2) :121-130

Tamborska E., J. Kotlinska, and R. Langwinski Ethanol-Opioid Interaction in Mice. Polish Journal of Pharmacology and Therapeutics. 36 :337-344.

Taylor, A.N., L.R. Nelson, B.J. Branch, N. Kokka, R.E. Poland (1984) Altered Stress Responsiveness in Adult Rats Exposed to Ethanol in Utero: Neuroendocrine Mechanisms. CIBA Foundation Proceedings 105 :47-65.

Testar, X., M. Llobera, and E. Herrerra (1988) Comparative Metabolic Effects of Chronic Ethanol Intake and Undernutrition in Pregnant Rats and their Fetuses. Alcoholism: Clinical and Experimental Research 12 :197-200.

Vathy, I.U., A.M. Etgen, R.J. Barfield (1985) Effects of Prenatal Exposure to Morphine on the Development of Sexual Behavior in Rats. Pharmacology Biochemistry and Behavior 22 :227-232.

Viglieccа, N.S., H. Ferreyra Moyano, and J.C. Molina (1986) Acute Prenatal Ethanol Exposure in Rats: a Behavioral Study Acta Physiologica Pharmacologica Latinoamericana 36 :463-472.

Wagner, G.C., M.F. Jarvis, D.S. Gottesfeld, M. Giorlando, J. Rabii (1986) The Effects of Prenatal Morphine on the Responsiveness to Morphine and Amphetamine. Pharmcology Biochemistry and Behavior 24 :757-759, 1986.

Zagon I.S., and P.J. McLaughlin (1982) Analgesia in Young and Adult Rats Perinatally Exposed to Methadone. Neurobehavioral Toxicology and Teratology 4 :455-457.

Zbinden, G. (1981) Experimental Methods in Behavioral Teratology. Archives of Toxicology 48 :69-79.

Zimmerberg B., and E.P. Riley (1986) Side-Preference in Rats Exposed to Ethanol Prenatally. Neurobehavioral Toxicology and Teratology 8 :631-635.

Appendix I

Developmental Test Battery

Note: The numbers in parentheses represent the postnatal day at which the assessment of the characteristic began.

Physical Development

Pinna Detachment: Both pinna were completely detached from the head. (2)

Incisor Eruption: Both upper and lower incisors were clearly visible above the gum line. (6)

Primary Coat: The beginning of white, downy-like, fur covering was clearly visible. (8)

Eye opening: both eyes were completely open. (10)

Reflex Developmnet

Surface Righting: the animals were required to return to an upright position within three seconds after being placed on a supine position, three consequtive times. (2)

Negative Geotaxis: the animals were required to complete a 180° turn within 30 seconds after being placed on the incline plane facing downwards, for three consecutive times. During trials the animals were kept for 5 seconds on a horizontal position. (10)

Spontaneous Behavior

Crawling: the animals were required to demonstrate forward motion without trunk elevation, equivalent to the length of the shoebox cage (12 cm). (7)

Walking: the animals were required to walk for approximately 20 cm on a countertop. Walking was demonstrated by forward motion of the trunk supported by all limbs. (7)

Sexual Development

Testes Descent: the testes were visible as permanent protrusions and had developed into scrotum. (25)

Vaginal Opening: an unobstructed orifice was apparent when slight pressure was applied under the tail. (25)