PRE- AND POSTNATAL EFFECTS OF NICOTINE ON THE DEVELOPMENT AND BEHAVIOR OF RATS*

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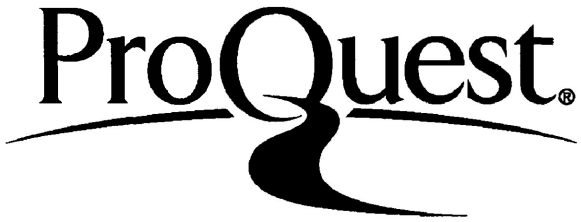
Running head: **PRENATAL NICOTINE EFFECTS**



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ABSTRACT

The effects of nicotine administered during one of three gestational trimesters on development and behavior of rats were investigated. Pregnant female SHS (Satinder's Heterogeneous Stock) rats were injected subcutaneously with nicotine (0.0, 0.3 or 0.6 mg/kg/day) during one of three gestational trimesters (days 0-6, days 7-13, or days 14-20). No differences were found between groups in litter size, litter weight, male-female litter ratio or postnatal mortality. However, progressively later trimesters and higher nicotine dosages retarded the appearance of developmental signs and reflexes. At 29 days of age, one day after weaning, emergence from home cage was measured. Rats prenatally exposed to 0.3 mg/kg/day of nicotine and rats exposed during the second trimester (7-13) had shorter emergence latencies than the other groups. Following emergence testing, all animals were exposed to the open field for 4 successive days. The 5-minute session each day revealed higher defecation scores and increased activity in the group prenatally exposed to 0.3 mg/kg/day of nicotine. Animals prenatally treated during the third trimester, defecated

significantly more than animals treated during the first trimester. On completion of open field testing, each rat was examined for an unconditioned escape response to electric shock. No differences were found between groups when litter differences were taken into account. After one day of rest, animals were given 4 days of either-way avoidance training. Following training, the effects of postnatal nicotine administration were assessed by administering nicotine (0.0, 0.2, 0.3 or 0.4 mg/kg counterbalanced over 4 days) 25 minutes before avoidance testing. Rats prenatally exposed to nicotine during the second trimester displayed superior avoidance acquisition and avoidance performance during the postnatal nicotine challenge, but this difference became non-significant when the differences between litters were considered. Differences in one-way avoidance performance with the different postnatal nicotine doses were only observed for two groups: animals exposed to saline during the first trimester and animals exposed to 0.3 mg/kg/day during the third trimester. In both of these groups, the postnatal nicotine dose most comparable to the dose received prenatally resulted in the best avoidance performance. The effects of pre- and postnatal nicotine on development and behavior and the presence of greater susceptibility to a specific

nicotine dose or gestational period is discussed. It can be concluded that different nicotine dosages administered during different periods of gestation can differentially affect development and behavior.

Pre- and Postnatal Effects of Nicotine on the Development and Behavior of Rats

Although the behavioral effects of nicotine have been studied extensively and several studies have examined teratogenic effects of nicotine administered prenatally, very little research has been aimed at investigating the behavioral effects of prenatal nicotine.

Considering increasing suggestions from human studies that smoking during pregnancy may be implicated in childhood hyperactivity and decreased cognitive functioning, further investigation of the behavioral effects of nonmorphological prenatal nicotine dosages is warranted. Of the limited number of studies that have examined behavioral prenatal effects, numerous methodological flaws and inconsistencies are present. In particular, virtually no consideration has been given to drug dosage or to the developmental stage of the organism at the time of prenatal nicotine administration despite indications that failure to take these factors into account may result in varying outcomes. The aim of the present study was, therefore, to systematically

investigate the behavioral effects of three doses of nicotine administered prenatally during different gestational periods.

A secondary aim of this study was to investigate the interactive effects of pre- and postnatal nicotine on avoidance performance in rats. Therefore, literature on postnatal behavioral effects of nicotine is also presented.

Behavioral Nicotine Effects

Nicotine affects cholinergic neurotransmitter systems both in the central and peripheral nervous system. This drug readily crosses the blood-brain barrier and concentrates in the molecular and pyramidal cell layers of the hippocampus where it has the general effect of increasing arousal (Hubbard and Gohd, 1975). Peripheral effects include respiratory stimulation and blood pressure elevation. Nicotinic cholinergic receptors are stimulated with low doses of nicotine whereas with high doses, they are initially stimulated and then inhibited (i.e. desensitized). This drug has also been known to cause release of amine transmitters

including noradrenaline, serotonin and dopamine (Taylor, 1980). The physiological and behavioral responses to nicotine are multiple and varied because of its multisite action and multiple effects.

Nicotine may stimulate or depress spontaneous motor activity in rats depending upon dosage, mode of drug administration, duration of drug exposure, sex of the animal and genetic strain (Marks, Miner, Cole-Harding, Burch and Collins, 1986; Silvette, Hoff, Larson, and Haag, 1962).

In low doses (0.2 mg/ml saline/kg) nicotine base (from nicotine hydrogen tartrate, BDH, England) stimulates spontaneous locomotor activity without affecting exploratory efficiency (Fitzgerald, Oettinger and Bättig, 1985) whereas, in large doses (0.8 mg/kg), it has a depressing effect (Silvette et al., 1962).

Tolerance to the EEG arousal and activity enhancing effects of nicotine occur rapidly (Hubbard and Gohd, 1975). The mode of nicotine administration affects the time required for such tolerance to develop. When nicotine is received via osmotic minipumps rather than via injection, tolerance to the effects of nicotine

occur more rapidly (Cronan, Conrad and Bryson, 1985).

Females tend to be more affected by nicotine than males (Cronan, Bryson and McNair, 1984; Cronan et al., 1985).

The effects of nicotine on locomotor activity of rats are also influenced by genetic factors (Marks et al., 1986). Rat lines with high baseline activity are affected more markedly than are lines with lower baseline activity (Bättig, Driscoll, Schlatter, and Uster, 1976; Schlatter and Bättig, 1979).

The extensive literature of nicotine's effects on animal learning has established that it has both facilitating and impairing effects on learning and memory in animals. Among other variables, nicotine's effects depend on dose, task, and species.

Accumulated evidence shows that relatively small doses of nicotine can enhance performance on learning and memory tasks, irrespective of whether the drug is administered before or after learning (Bättig, 1970; Garg and Holland, 1969), whereas, larger doses appear to have a depressing effect.

Haroutunian, Barnes and Davis (1985) found that

passive avoidance retention was facilitated by 0.05 and 1.0 mg/kg of nicotine (95-98% free base, Sigma Chemical Co.), but not by saline, 0.1 or 0.5 mg/kg of nicotine. They have suggested that the lower dose (0.05 mg/kg) of nicotine may have an Ach releasing property, while the higher dose (1.0 mg/kg) acts on the post-synaptic receptors directly.

Fleming and Broadhurst (1975) found no effect of subcutaneous injections of saline or five nicotine hydrogen tartrate doses (0.05, 0.1, 0.2, 0.4 or 0.8 mg/kg or 0.016, 0.03, 0.06, 0.12 or 0.25 mg/kg of nicotine expressed as base, respectively) on two-way escape avoidance conditioning in the RHA or RLA rats.

There is some support (Erickson, 1971) that small doses of nicotine alkaloid (0.1 and 0.4 mg/kg, 95-98% pure, Sigma Chemical Co.) enhance avoidance acquisition by permanently facilitating the consolidating neural memory trace and not merely by stimulating performance. These studies further show that nicotine-trained rats do not require the drug to maintain performance and, therefore, no drug dissociation state exists. Contrary to this finding, Hall and Morrison's (1973) experiments

suggest that rats that have learned to avoid while under the influence of nicotine become dependent on the drug for effective performance, and that this effect is related to the stressfulness of the situation (Morrison, 1974a), since a further study (Morrison, 1974b) showed that warning or feedback signals reduced or eliminated this nicotine dependence. To further complicate the issue, disruptive effects of post trial nicotine have also been found. In mice, low doses (1.0 mg/kg) of nicotine for C57 females and 2.0 mg/kg of nicotine (98% pure, Kodak Laboratory Chemicals) for DBA males and females administered during the relearning phase were found to block retrieval of previous learning and, therefore, inhibit relearning of the active avoidance task (Gilliam and Schlesinger, 1985). Garg and Holland (1969) also reported disruptive effects of post trial nicotine (0.8 mg/kg nicotine hydrogen tartrate or 0.25 mg/kg nicotine, expressed as base) on shuttle box avoidance response in rats.

Only one study known to this author has looked at pre- and postnatal effects of nicotine on active avoidance. Persson (1984) found no effect of 0.0, 0.05,

0.1 or 0.2 mg/kg of postnatal nicotine (98% pure, BDH Chemicals Limited) on avoidance performance of rats prenatally treated with nicotine (0.3 mg/kg), or saline.

Teratogenic Effects of Nicotine

Various complications associated with pregnancy, increase with maternal smoking. These include increased fetal distress, increased probability of spontaneous abortion, reduced birth weight and long-term growth, increased perinatal death rate, and increased neonatal morbidity (Abel, 1980). Although the mechanisms by which nicotine exerts its teratogenic effects have not clearly been elucidated, its vasoconstricting effect on the maternal blood supply to the placenta, resulting in an impairment of uteroplacental circulation has been implicated in retarded fetal growth in the offspring of heavy smokers (Mochizuki, Maruo, Mosuko, and Ohtsu, 1984).

Besides, increasing complications associated with pregnancy, behavioral effects of smoking during pregnancy have also been suggested in human studies. Newborn infants born to women who smoke have been

reported to perform significantly worse on the operant tasks of head turning and sucking (Martin, 1977). Smoking during pregnancy has also been implicated in childhood hyperactivity (Denson, Nanson, and McWalters, 1975). In addition, a decrease in Motor Scale scores, and a decline in verbal comprehension and fine motor skills (Gusella and Fried, 1984), and significantly lower scores on Block Design and Vocabulary subtests of the Wechsler Intelligence Scale for Children, which are considered to be meaningful subtests of cognitive functioning (Dunn, McBurney and Sandraigram, 1977), were found. On the contrary, no relationship was found between mental or psychomotor development scores and smoking during pregnancy in 8 month old infants (Streissguth, Barr, Martin, and Herman, 1980). This discrepancy may be the result of using different measures, or it is possible that adverse effects are only manifested at a later age.

The effects of intrauterine exposure to nicotine on behavior can be studied using animal models under controlled conditions which are simply not possible in humans.

Although subcutaneous administration of nicotine (Eastman Kodak reagent, 98% pure), 0.5-5.0 mg/kg/twice daily to rats through pregnancy has been reported to increase gestational length, reduce litter size and birth weight, and increase postnatal mortality (Becker, Little, and King, 1968; Becker and Martin, 1971), no such effects were found by Abel, Dintcheff, and Day (1979) when rats were injected with lower doses of nicotine (Sigma Chemical Co., 1.5 mg/kg/day), throughout pregnancy. Abel et al.'s (1979) study did not test for behavioral deficits which may have been a more sensitive measure of teratogenicity at this lower dose.

Several studies have found behavioral and/or cognitive deficits, but available data are conflicting and confusing, probably due to variation in methodology and dosages.

Nicotine injected (3 mg/kg/twice daily) to pregnant guinea pigs produced offspring that were severely impaired behaviorally. These animals, as adults, were found to be deficient on spontaneous alteration, black-white discrimination, and reversal (Johns, Louis, Becker, and Means, 1982).

Baer, McClearn, and Wilson (1980) found that offspring of mice prenatally treated with tobacco smoke were less likely to survive to weaning and displayed depressed open field activity at 28 and 50 days of age.

Martin and Becker (1970) found that male rats whose mothers were injected twice daily throughout gestation and nursing with 3 mg/kg/day of nicotine (Eastman Kodak reagent - quality pure nicotine in a saline buffer) were significantly more active on the Wahmann activity wheel, than saline controls. The offspring of mothers receiving the same dose of nicotine only through gestation differed little from the controls.

Male offspring of rats which had received either daily nicotine injections (0.6 mg/kg/day of Eastman Kodak reagent - quality pure nicotine in a saline buffer) or hypoxic episodes throughout gestation and the nursing period performed more poorly on the fixed ratio, variable interval, discrimination, and discrimination-reversal schedules (Martin and Becker, 1971).

A study by Bertolini, Bernardi, and Genedani (1982) failed to find any effect of prenatal exposure to nicotine (0.3 mg/kg/day prepared daily from concentrated

nicotine, 98%, BDH), on avoidance behavior in adulthood. But another study (Genedani, Bernardi, and Bertolini, 1983) found a sex-linked difference in avoidance learning in offspring treated with a higher nicotine dose (0.5 mg/kg/day of 98% pure nicotine, BDH). The acquisition of the avoidance response was impaired in the males.

Peters and Tang (1982) also found sex-dependent changes following prenatal nicotine exposure in the rat. Nicotine treatment reduced the number of male pups born and male body weight at birth. Rearing activity and horizontal locomotor activity were also depressed, but in males only.

In another study, male offspring of nicotine treated (nicotine tartrate received via osmotic minipumps) rats exhibited a high degree of saccharin preference, typically only present in normal females. Nicotine treatment resulted in a complete suppression of the rise in male plasma testosterone, which is characteristic of gestational day eighteen. Therefore, the sexual dimorphism in this behavior (saccharin preference) was found to be abolished (Lichtensteiger

and Schlumpf, 1985).

Prenatal exposure to nicotine was also found to differentially affect rats of different genetically-selected lines (Persson, 1984). Behavioral effects of 0.3 mg/kg/day of nicotine were examined in the MNR, MR (genetically selected for low and high open field defecation respectively) (Broadhurst, 1960), the RHA, RLA (selected for high and low rates of active avoidance respectively) (Bignami, 1965), and the SHS (a control line developed by a 4-way cross of the above mentioned genetic lines) rats (Satinder, 1980). Only the RLA rats showed depressed open field activity after prenatal nicotine exposure. Suppression of avoidance learning was only evidenced in the SHS and RLA lines.

The findings that cigarette smoke reduced whole brain and cellular weight at 21 days postnatally and cellular loss in brain up to 120 days postnatally (Barnes, King, Goldberg, and Harris, 1981), suggest that behavioral and cognitive deficits may exist but are not being tapped by present tests.

The term, behavioral teratology, is used to describe deleterious changes in the behavior of animals

(rather than morphological changes) attributed to teratogenic agents administered during prenatal development (Coyle, Wayner, and Singer, 1976; Leonard, 1982). Since behavioral effects occur at dose levels lower than those producing gross malformations, and when the agent is administered at times other than that optimal for central nervous teratogenesis, behavioral testing can be a sensitive and relevant technique for detecting adverse consequences of prenatal exposure to drugs and chemicals to the developing nervous system (Butcher, 1976), and a central nervous system deficit may only become evident upon a specific kind of behavioral change (Spyker, 1975).

Behavioral tests are also considered to be a sensitive evaluation technique because the use and integration of several primary systems can be summarized in one behavioral measure.

The sensitivity of behavioral tests could be used in a way that would provide direct information about low levels (closer to human exposure levels) of drugs not causing gross morphological defects (Wilson, 1975).

Larsson and Hard (1982) feel that a behavior which

is biologically relevant for an animal is more likely to reveal the relationship between the behavior and its substrate than are nonspecific behaviors.They advocate describing alterations occurring in the normal course of maturation of different species-specific behaviors such as maternal and sexual behavior. Sensory and motor functions should also be assessed by observing locomotion, and reflexes such as body righting. Rodier (1976) cautions against using a large battery of different tests since this will probably introduce so many new variables that a subtle behavioral effect may not be recognized with certainty.

The ideal experimental strategy for the evaluation of potential behavioral teratogens has not yet been identified (Zbinden, 1981) but must include a small number of tests which can quantify a broad spectrum of behavior characteristics and, at the same time, involve minimal manipulation and training of animals.

The appearance of certain physical features in the rat can serve as indicators of pre- and postnatal adversities and involve minimal handling of animals. For example, fur development and eye opening may be retarded,

when mothers are undernourished (Smart and Dobbing, 1971). Furthermore, delays in the development of certain kinds of behavior could be a more sensitive indicator of behavioral teratogenic effects than tests of adult behavior (Coyle et al., 1976).

Both open field and avoidance testing have been shown to be effective behavioral measures in detecting learning differences, due to prenatal drug exposure (Rodier, 1976).

Extraneous influences that can affect offspring behavior must be rigorously controlled since behavioral effects of prenatal drug exposure can be very subtle.

Nicotine and Different Gestational Periods

The effects of nicotine on the embryo can be more fully delineated by investigating the effects of this agent during different periods of gestation (Persaud, 1982).

The finding that chronic drug administration may mask activity which, in an acute dosage, at a susceptible period, would be teratogenic (Kalter, 1968; Wilson, 1975), warrants an investigation of the effects

of short term nicotine administration throughout pregnancy.

When administered repeatedly, certain chemicals such as mitomycin C and the well known teratogenic dye trypan blue, can influence the rate of their own metabolism (Wilson, 1975). Some of the mechanisms by which this may occur include induction of catabolizing enzymes (microsomes) by the liver or other tissues, inhibition of naturally occurring enzymes which degrade chemicals, or impairment of function in important maternal homeostatic organs such as the liver, or kidney. The agent could, therefore, be appreciably raised or lowered as a consequence of such changes in metabolism or distribution. These changes may occur within 3 or 4 days after the beginning of repeated treatment.

Studies which have administered nicotine through the entire gestation period may have highly variable results since adaptive changes in maternal homeostatic systems may change the dosage reaching the embryo.

Consequently, maternal metabolic changes causing variations in dosage to the developing organism would be

avoided by employing short term treatment periods.

During the embryonic and fetal period, particular organs and parts are developing at their own paces, each with their own characteristic temporal shift in susceptibility to injury or critical period (Katler, 1968).

The developmental stage determines which tissues or organs will be affected at any given time (Wilson, 1964; Wilson, 1973). Therefore, behavioral teratogens may produce specific anomalies dependent upon the developmental stage at the time of drug administration (Coyle et al., 1976; Leonard, 1982; Vorhees, Brunner, and Butcher, 1979). For example, Gatling (1964) found that 12-50 mg of nicotine produces cephalic lesions in 10-11 day old chicks but not in 13 day old embryos.

Many studies have failed to consider the developmental stage of the organism at the time of prenatal nicotine administration or have only examined one short period in gestation and have, therefore, provided limited and/or conflicting information (Coyle et al., 1976). Nishimura and Nakai (1958) found that malformation rates and lethality were higher in mice,

when nicotine (25 mg/kg/day) was administered during gestation days 9-11. In contrast to this finding, Geller (1959) found no effect on viability or frequency of malformations in rats administered nicotine (1.5-4.5 mg/kg/day) during gestation days 9-12 or 1-20.

Hammer and Mitchell (1979) found that when nicotine was administered to rats during the initial 5 days of pregnancy, the rate of embryonic cell proliferation was markedly reduced, but organogenesis and weight at birth were not affected.

Lindenschmidt and Persaud (1980) injected pregnant rats with a single dose of nicotine (5 mg/kg, Eastman Kodak Co.) on day 9 of gestation with no significant adverse effects on embryonic and fetal viability.

Two studies that do consider the developmental stage at the time of nicotine administration implicate the latter part of gestation as the period of greatest vulnerability.

One study found a significant shortening of the gestation period in mice exposed to higher doses of nicotine hydrogen tartrate (BDH) (2,700 μg/kg/day, expressed as free base) during the second and third

trimesters (Nasrat, Al-Hachim, and Mahmood, 1986).

Another study (Al-Hachim and Mahmood, 1985) examined how the trimester of pregnancy during which 900, 1,800 or 2,700 μg/kg/twice daily of nicotine hydrogen tartrate (expressed as base) might influence maturation and development of the CNS in mice as assessed by audiogenic seizures. Prenatal nicotine prolonged the latency and delayed the onset and extinction of audiogenic seizures in the offspring. These effects were more pronounced in the third trimester, particularly with the highest nicotine dose.

None of the above studies examined behavioral deficits which may be a more sensitive indicator of teratogenicity.

Presently, there appears to be no agreement as to whether there is a critical period during gestation when fetal growth and development are maximally affected by smoking and/or factors related to smoking (Abel, 1980). The most vulnerable periods with respect to behavioral deficits have also not yet been delineated (Coyle et al., 1976).

Rodier (1976) reviewed several studies examining

critical periods and noticed that behavioral teratogens are so dependent on the time of administration that many studies show very similar effects of a wide variety of teratogens when the time variable is controlled.

During cleavage and the early germ layer stages, the embryo is resistant to malformations. At this predifferentiation stage, an adequate stimulus would probably result in death or retardation of growth.

During organogenesis, the embryo becomes susceptible to most teratogenic agents which may produce their highest incidence of malformations.

The fetal period is occupied by histogenesis and functional maturation together with the growth required to achieve body size as encountered at birth. Kretchman (1973) found that intrauterine growth retardation was a result of environmental stress in this part of gestation. Interference with development during this fetal period may also result in functional disturbances (Wilson, 1973).

Considering the importance of the developmental stage at the time a drug is administered, it seems apparent that nicotine should be administered at various

periods during gestation. Short term nicotine administration could provide information about whether acute exposures will produce behavioral effects postnatally and whether administration at some points in gestation is more probable than at other points in producing these effects.

Minimal doses must be used to detect periods of greatest susceptibility since larger doses may cause deficits to appear on days other than those of greatest susceptibility.

By associating the functional deficit with the stage of development when exposed, more insight may be gained into the mechanism of nicotine's action on the developing organism (Spyker, 1975).

Stanton (1978) expresses reservations about identifying critical periods during prenatal and perinatal periods since physiological and behavioral changes in the adult organism which signal such effects are usually subtle. But, as discussed earlier, behavioral testing should be a sensitive measure.

The primary purpose of the present study was to examine the teratogenic effects of nicotine by employing more sensitive teratogenic measures: (development and

behavioral measures rather than morphological) while adding clarity to previous research by considering both the stage of development at the time of nicotine administration and drug dosage.

To this end, the present study was designed to assess how administration of nicotine during different periods of gestation (day 0-6, day 7-13, or day 14-20) with different nicotine doses (0.0, 0.3 or 0.6 mg/kg/day) might influence physical maturation, development of reflexes, spontaneous movements, open field activity, avoidance behavior and sexual development. The presence of behavioral effects and developmental delays, and whether greater susceptibility was present at some periods than at other periods in producing these effects, was examined.

Method

Part I: Prenatal Treatment and Reproductive Performance Maternal Animals

Nineteen experimentally naive SHS (Satinder's Heterogeneous Stock) (Satinder, 1980) female rats were used.

The laboratory in which mothers were housed was maintained at 22 $\pm$ 1°C and the humidity level maintained at 40%. Fluorescent lighting was present from 8:00 a.m. to 8:00 p.m. each day. Food and water were provided ad libitum.

Experimental Design

Genetic background of maternal animals was controlled by selecting six groups of 3 litter-mate females. In three of the groups, litter-mate females were randomly assigned to each dosage condition and in the other three groups, litter-mate females were randomly assigned to each gestation period.

To investigate whether or not the period of drug administration had different effects on development and behavior, the gestational period was divided into 3 sections; roughly corresponding to the cleavage and early germ layer stage (days 0-6), organogenesis (days 7-13), and the fetal period (days 14-20). These time periods were also selected because the few studies that have examined short term exposure effects of nicotine fall into one of these gestational trimesters.

Different drug dosages were administered during each one of the 3 gestational periods to study the

effects of dosage during the three periods. Drug dosage conditions comprised of either subcutaneous injections of 0.0 mg/kg/day of nicotine (a saline solution to control for the stress involved in this mode of drug administration), 0.3 mg/kg/day of nicotine or 0.6 mg/kg/day of nicotine (98% min free base, BDH, Poole, England). See Appendix A for nicotine composition. One-third of the drug dose was administered in the morning at 9:00 a.m. and the remaining two-third was administered in the evening at 5:00 p.m. each day (8 hr and 16 hr intervals, respectively) for all 3 dose conditions. This was done to maintain a relatively equivalent drug concentration over a 24 hr period. The 0.3 and 0.6 mg/kg/day nicotine doses were chosen empirically from a previous dose-response study conducted in this lab to ensure doses with minimal morphological impact. The experimental design was, therefore, a 3 (Dosage) x 3 (Gestational Period) factorial design with a minimum of 2 animals per factorial cell.

Previous prenatal studies have often employed paired feeding as a control for the effects of possible variations in food or fluid consumption during gestation

due to the effects of nicotine administration. This procedure involves daily monitoring of the food consumed by animals being treated with nicotine, and during the next 24 hrs, providing the same amount of food to non-treated control animals. But, this delay of matching food for the control group by 1 day may have an effect at critical stages of the pregnancy. Furthermore, this control neglects the size of the unborn litter. Food and water restrictions may affect the availability of nutrients to fetuses in larger litters. For the purposes of this study, an alternative control was implemented. The possible variations in maternal consumption during gestation were controlled by measuring daily maternal body weight, food consumption and water intake during gestation. These measures were used as covariates to have a statistical control when evaluating litter size and litter weight at birth (Satinder, 1985).

Although the suggestion has been made that surrogate fostering should be employed to control for possible alterations in maternal behavior and for possible effects of nicotine transmitted to the pups during lactation, this control was not used for practical and ethical reasons (Satinder, 1985).

Since nicotine administration was discontinued prior to parturition and since 80-90% of nicotine is metabolized in the liver and excreted mainly via the kidney within 24 hours after administration, it was felt that the amount of nicotine secreted in the milk received by offspring prenatally treated with nicotine during the 3rd trimester was negligible with regard to development and behavior (Al-Hachim and Mahmood, 1985). For this reason, cross fostering was not employed.

Instead, maternal care was controlled for by using mother of litter as a covariate in the statistical analysis of the developmental and behavioral data; offspring body weights at birth and at regular intervals up to the time of weaning were monitored to account for possible changes in milk production on postnatal development (Satinder, 1985).

Procedure

Each female was paired with a SHS male in a suspended mesh cage and allowed to mate overnight. Each morning after pairing, all females were checked for the presence of vaginal plugs (day 0 of pregnancy). On day 0 of pregnancy, the male was removed from the cage and the gravid rat was randomly assigned to 1 of 3 dose

conditions in one of 3 gestational periods (see experimental design above). Animals were assigned according to gestational period and prenatal drug dosage to ensure equal representation of animals in each experimental group.

The maternal animal's food and water intake and weight gain were monitored daily from day 0 of pregnancy to day 20 of pregnancy. The mothers were disturbed twice daily (at 9:00 a.m. and at 5:00 p.m.) for administration of subcutaneous nicotine injections only during one of the 3 gestational trimesters. On day 20, the gravid rats were placed in breeding cages and checked daily for litters. Litter size and offspring body weight at birth and at regular 7 day intervals up to the time of weaning (day 28) were also measured. Any dead pups were preserved for later autopsy examination.

Part II: Developmental Signs and Reflexes

Offspring

Offspring represented each of the 9 experimental groups described in Part I. All litters were coded so

that the experimenter was unaware of the prenatal treatment each group received. The laboratory was maintained under the same conditions described previously in Part I.

Experimental Design

The design was a 3 (Dosage) x 3 (Gestational Period) x 2 (Sex) factorial with a minimum of 3 animals per cell.

Age of appearance of the following common physical signs were noted: pinna detachment, primary coat, incisor eruption, development of fur, ear opening, eye opening and sexual development (testes descent in males and vaginal opening in females). Refer to Appendix B for physical signs and their approximate appearance in rats. Ages of appearance of the following reflexes were also observed: righting reflex, cliff avoidance, palmar grasp and visual placing (see Appendix C). These reflexes were chosen because they provide information about neuromuscular and sensory function; their presence or absence can be clearly and easily recognized; and minimal manipulation is required in their elicitation.

Procedure

Animals were examined at approximately the same time each day. Possible delays in the appearances of physical signs were monitored by noting the day each sign appeared in each rat. The time of appearance of the physical features were recorded according to the following criteria:

Pinna detachment. Unfolding of the pinnae of both ears to the fully erect position.

Primary coat of downy hair. Initial presence of downy hair.

Incisor eruption. Eruption of the upper incisors.

Development of fur. Initial presence of fur.

Ear opening. Presence of opening in both ears.

Eye opening. Complete uncovering of the membrane of both eyes.

Sexual development in females. Sexual maturation was determined in female rats by examining each female daily (starting day 28) within 1 hour of the start of the laboratory day cycle (8:00 a.m.-9:00 a.m.). The animals were weighed daily and vaginal orifice was examined for the rupture of the vaginal membrane. When slight pressure under the tail revealed an open vagina, the animal's age was recorded (Satinder, 1984).

Sexual development in males. Males were examined at the same time as females starting at day 28. The male rats were weighed and examined for descended testes. Age and body weight at descent of testes were recorded.

Each rat was also tested a few days prior to the expected appearance of each reflex until the day of appearance was noted. Appearance of reflexes were observed as follows:

Righting reflex. Each pup was placed on its back on a flat surface. Time taken to turn over on its ventral side was recorded. The cut-off time was 1 minute.

Cliff avoidance. Each pup was placed on the edge of the counter top with forepaws and face over the edge. The time taken by the rat to move away from the edge by withdrawing head and both forefeet was measured. The cut-off time was 1 minute.

Palmar grasp. This reflex was considered to be present when digits flexed to grasp a paper clip when palm of forepaw was stroked gently with the paper clip.

Visual Placing. Each pup was held by its tail with vibrissae not touching the surface. If the head lifted and forelegs extended in the direction of the surface, this reflex was considered to be present.

Part III: Postnatal Behavioral Testing

Offspring

One hundred and thirty-three naive pups prenatally exposed to one of the nine experimental conditions (see Part I) were used. At 28 days of age (the day of weaning), all pups were coded and housed individually. Laboratory conditions were maintained in the same way as described in Part I of the study.

Experimental Design

The design was a 3 (Dosage) x 3 (Gestational Period) x 2 (Sex) factorial with a minimum of 3 animals per cell. On day 29, one day after weaning, animals were tested for emergence from home cage. Animals were then tested for 4 days in the open field; 1 day for unconditioned escape response (UER) followed by 1 day of rest, 4 days of avoidance training and 4 days of avoidance performance under the effects of postnatal nicotine.

The emergence from home cage procedure was employed to provide information about spontaneous movement in a familiar surrounding whereas open field testing assessed activity in an unfamiliar environment. Either-way

avoidance training was selected as a behavioral measure to assess prenatal nicotine effects on acquisition of avoidance learning and to assess the effects of postnatal nicotine on established avoidance responding. In past research, the above behavioral measures have been successfully used in detecting differences due to prenatal drug exposure (Rodier, 1976).

Apparatus

Open field apparatus. The open field was 90 cm on each side with 45 cm high walls and is divided into 16 equal-area square sections marked on the floor. The front wall was a sliding door of transparent Plexiglas which served as an observation screen and as an entry for cleaning of the open field floor. Four 90 cm long fluorescent lights located 90 cm above floor level provided an illumination of 230 ftc (2,476 lx). White noise (65 dB.) was produced by a generator (sound intensity was measured at floor level with a General Radio sound-level meter) (Satinder and Hill, 1974).

A start box (22.5 cm x 22.5 cm x 15 cm) was attached to the center of the right wall adjacent to the field. The start box was separated from the field by a clear plastic sliding partition.

The unconditioned escape response and avoidance training apparatus. The apparatus consisted of a circular Plexiglas runway. This runway was 12 cm wide, 15 cm high and had an outside circumference of 220 cm. Guillotine doors divided the apparatus into four equal compartments. The floor consisted of stainless steel rods (0.25 cm diameter) spaced 1 cm apart. A scrambled shock (Lafayette Instrument Co., Lafayette, Indiana, Mastershock 82404) could be delivered to the grids. A speaker was located in the center of the circular runway (sound intensity is measured at floor level above the standard reference level of 0.0002 μ bar by a General Radio soundlevel meter, type 1551-C). Durations of electric shock were controlled by an automatic timer. A digital clock was used to record response latencies (Satinder, 1981).

Procedure

Animals were weighed daily through the entire testing period.

Spontaneous movement. The pups were allowed one day after weaning for adaptation to the individual cage before testing began. The next day (29 days old) spontaneous movement was measured through a procedure

called emergence from home cage (Ader and Conklin, 1963). The cover of the home cage was removed and the time for the rat to emerge from the cage with the head above the top of the cage was recorded. The cut-off time was 5 minutes.

Open field activity. One day after the animals were tested for spontaneous movement, they were tested in the open field. Each animal was taken from the experimental room and placed in a start box with the door of the field closed. As the door was lifted, both illumination and sound stimuli were turned on. Latency to enter the field, sections crossed, frequency of defecations in the box and in the field, number of entries into the box, crossing of center squares, and time out of the box were measured during each 5-minute trial on four consecutive days (at approximately the same time each day). The field was cleaned after every trial.

Unconditioned escape response (UER). Since different prenatal treatments may influence the UER's of the different groups, an estimate of the UER of each rat was desirable to detect such differences. The UER also affects avoidance acquisition (Satinder, 1976) and, therefore, serves as a control. All animals were tested

for UER the day following open field testing. Each animal was individually adapted to the circular runway for a 1-minute period before receiving a shock from the floor grids. Electric shock was administered until a UER had occurred. A UER was operationally defined as an animal running a quarter length distance of the runway (all doors open) either way within 5 seconds from the onset of the unconditioned stimulus (US). If the animal did not run within 5 seconds, the shock level was increased by 0.1 ma for each successive trial until the animal escaped within 5 seconds as described above. Each animal was given 10 trials. The intertrial interval was approximately 5 seconds (Satinder, 1981).

Avoidance training. In either-way avoidance training, rats must choose between running towards or away from the compartment where the conditioned and/or unconditioned stimulus was last presented. Since two-way responses are more complex than one-way responses, inferences may be made regarding response complexity in this type of avoidance task (Satinder, 1977). An avoidance trial included a maximum of 10 seconds of conditioned stimulus (CS) alone followed by 10 seconds of both CS and unconditioned stimulus (US) and a 40

second intertrial interval (ITI). The CS was a 70-dB, 9kHz pure tone against a background noise of 40dB. The shock intensity equivalent to the UER of each respective animal was used as US level in avoidance training. The CS was followed by the US, if no avoidance response occurred within the CS duration. The CS was terminated immediately following an avoidance response, and both CS and US were terminated immediately following an escape response. If an animal did not escape within 20 seconds of stimulus exposure, that specific trial was recorded as a "no escape" response. The first training trial was an escape trial for each rat. Each rat was given 10 acquisition trials each day for four consecutive days.

On the first trial each day, the animal was restricted to a one-way response (counterclockwise direction). The door permitting a clockwise response was closed so that on subsequent trials the rat was running towards or away from the compartment in which the tone and/or shock was last presented. For the next nine trials, the animal was permitted to run either-way and the direction of each response was recorded (Satinder, 1977). The crossing response was made very distinctive

to each rat by manually adjusting the height of the guillotine doors so that the rat was required to just squeeze under the door to pass.

After 4 days of avoidance training, each rat was exposed to 4 days of avoidance testing 25 minutes after receiving nicotine (0.0, 0.2, 0.3, or 0.4 mg/kg, 98% min free base, BDH) injected subcutaneously each day. By random assignment, dosage order was determined and held constant for all animals. Animals were assigned according to gestational period and prenatal drug dosage to ensure equal representation of animals beginning the drug rotation at each dose.

The number of avoidances, avoidance latencies, escape latencies, and frequency of intertrial crossings were recorded for both avoidance during training and under the effects of nicotine.

Results and Discussion

Results were analyzed by analysis of variance and when appropriate, by analysis of covariance. In situations where there was lack of homogeneity, nonparametric tests were used and both chi-square and F-values are given. Differences with associated probabilities less than 0.01 were considered

significant, but for a better perspective probability levels less than 0.05 which added some specific relevance were also identified.

Part I: Prenatal Treatment and Reproductive Performance

Maternal Measures During Pregnancy

Maternal differences in fertility and body weights may result in varying outcomes of pregnancy and thus confound the effects due to nicotine exposure. To control for this, measures of body weight at pairing, body weight at day 0 of pregnancy, and number of days between pairing and day 0 of pregnancy were taken before random assignment of mothers to the treatment conditions. No differences in fertility or body weight at pairing or at day 0 of pregnancy were found between dams in the 9 experimental groups.

Previous research suggests that maternal food consumption decreases when dams are treated with nicotine (Wager-Srdar, Levine, Morley, Hoidal, and Niewoehner, 1984). Reduced maternal food and fluid consumption during gestation could affect postnatal development and function. Therefore, daily measures of maternal body weight, food consumption, and water intake

were assessed during the entire period of gestation for possible differences among the groups. These measures were also used as covariates for the pregnancy outcome measures.

Percent change in body weight over the entire gestational period did not differ between experimental groups, although the 2 mothers treated with dose 3 (0.6 mg/kg/day of nicotine) during the third trimester (days 14-20) gained relatively less weight than the other groups. (See Table 1 for cell means).

Insert Table 1 about here

Total maternal food consumption was also unaffected by gestation period or dosage level. Mean daily food consumption during the trimester in which a group was injected with nicotine, did not significantly differ from that of groups not receiving nicotine during the same trimester. See Table 2 for all means of average daily food consumption, during the trimester in which nicotine was administered .

Table 1

Percent Change in Body Weight (gm) over Gestation (day 0-day 20) in Rats (n=2) Receiving 1 of 3 Nicotine Dosages During 1 of 3 Trimesters

	Nicotine dosage (mg/kg/day)			
Trimester	0.0	0.3	0.6	MEAN
1	39.2	32.2	45.9	39.1
2	46.9	31.2	39.3	39.1
3	33.1	42.6	24.8	33.5
MEAN	39.7	35.3	36.7	

Insert Table 2 about here

Food consumption increased with advancing gestation. Less food was consumed by mothers receiving dose 2 (0.3 mg/kg/day) than dose 1 (saline) or dose 3 (0.6 mg/kg/day) across all gestational periods.

Total water intake was not different for the different experimental groups although there was a trend for reduced fluid intake with higher nicotine doses across all 3 trimesters. Refer to Table 3 for cell means.

Insert Table 3 about here

Table 2

Mean Daily Food Consumption (gm) During Drug Period in Rats (n=2) Receiving 1 of 3 Nicotine Dosages During 1 of 3 Trimesters

	Nicotine dosage (mg/kg/day)			
Trimester	0.0	0.3	0.6	MEAN
1	15.6	14.4	14.6	14.9
2	17.5	15.2	16.6	16.4
3	21.6	18.3	18.6	19.5
MEAN	18.2	16.0	16.6	

Table 3

Mean Daily Water (ml) Intake During Drug Period in Rats (n=2) Receiving 1 of 3 Nicotine Dosages During 1 of 3 Trimesters

	Nicotine dosage (mg/kg/day)			
Trimester	0.0	0.3	0.6	MEAN
1	34.5	27.2	26.0	29.2
2	46.5	33.5	29.1	36.4
3	41.9	41.7	39.5	41.0
MEAN	41.0	34.1	31.5	

Failure to find differences in maternal weight gain, food consumption and fluid intake among groups contradicts Wager-Srdar et al.'s (1984) findings, that short term (5 day) exposure to 2 and 4 mg/kg/day of nicotine (Sigma Chemicals) suppressed growth rate and food intake. But, the lack of differences found in these maternal measures is consistent with Grunberg, Bowen and Morse's (1984) finding that nicotine dihydrochloride (J.T. Baker Chemical Co.) administration (4, 8 or 12 mg/kg/day computed as base via miniosmotic pumps for 12 days) does not change consumption of laboratory chow or water. Since there was a trend in the present study for less food and water consumption with the higher nicotine doses, it may be possible that failure to find significant differences among groups can be attributed to the small size of the experimental groups (n=2). Although the present study used lower nicotine doses (0.0-0.6 mg/kg/day) than Wager-Srdar et al.'s (1984) study, failure to find differences in food consumption cannot be attributed to the use of lower doses since Grunberg et al. (1984) used higher doses (4-12 mg/kg/day) and failed to observe any differences. Both the present study and Grunberg et al.'s (1984) study

employed techniques to maintain a more constant concentration of drug. It appears, therefore, when nicotine is administered for a short period of time in a relatively constant dosage, (whether the dosage is low or high) food and water consumption will not be significantly affected.

Reproductive Performance

No differences were found among experimental groups in the size of litters born, number of litters surviving to weaning, length of gestation, or maternal mortality. These findings are inconsistent with other studies which have reported increases in gestational length, reduced litter size and birth weight, and increased postnatal mortality with subcutaneous administration of nicotine (0.5-5.0 mg/kg/twice daily) to dams, through the entire pregnancy (Becker, Little, and King, 1968; Becker and Martin, 1971). These findings, however, are consistent with Abel, Dintcheff, and Day's (1979) study which failed to find morphological effects using lower nicotine doses (1.5 mg/kg/day) throughout pregnancy. See Table 4 for cell means of reproductive outcome measures.

Insert Table 4 about here

The number of pups in the litter at birth, the number and average body weight of males and females in the litter at birth and at 7-day intervals until weaning were also examined for possible differences. Table 5 and Table 6 give the mean litter size and weight of the pups in the 9 experimental conditions at birth and at weaning, respectively.

Insert Table 5 about here

Insert Table 6 about here

Table 4

Reproductive Performance of Rats Administered 1 of 3 Nicotine Dosages (mg/kg/day) During 1 of 3 Trimesters

	Trimester 1[a]			Trimester 2[b]			Trimester 3[c]		
	Nicotine			Nicotine			Nicotine		
	0.0	0.3	0.6	0.0	0.3	0.6	0.0	0.3	0.6
Number of Litters Born Alive	2	2	2	2	2	2	2	2	2
Mean Number of Pup Stillbirths	1.5	0	0	0	0	0	0	0	0
Litters Surviving to Weaning	2	2	2	2	2	2	2	2	2
Length of Gestation (Days)	22	22	22	22	22.5	22	21.5	22	21.5
Maternal Mortality During Gestation	0		0	0	0	0	0	0	0
Total Number of Maternal Animals	2	3	2	2	2	2	2	2	2

Note: [a]Days 0-6. [b]Days 7-13. [c]Days 14-20

Table 5

Mean Number and Weight (gm) at Birth of Pups Prenatally Exposed to 1 of 3 Nicotine (mg/kg/day) Dosages During 1 of 3 Trimesters.

	Trimester 1[a]			Trimester 2[b]			Trimester 3[c]		
	Nicotine			Nicotine			Nicotine		
	0.0	0.3	0.6	0.0	0.3	0.6	0.0	0.3	0.6
Mean Litter Size	11.5	6.0	12.0	9.0	10.5	8.0	4.5	10.0	4.5
Mean No Females/litter	4.5	2.5	4.0	6.0	5.0	3.5	1.5	5.5	2.5
Mean No Males/litter	7.0	3.5	8.0	3.0	5.5	4.5	3.0	4.5	2.0
Mean Litter Weight	5.7	6.7	6.4	6.1	6.7	6.4	6.4	6.3	6.8
Mean Female Weight	5.5	6.6	6.1	5.9	6.5	6.3	6.3	6.1	6.9
Mean Male Weight	5.9	6.7	6.6	6.4	6.9	6.6	6.4	6.6	6.7

Note: [a]Days 0 - 6. [b]Days 7 - 13. [c] Days 14-20.

Table 6

Mean Number and Weight (gm) at Weaning of Pups Prenatally Exposed to 1 of 3 Nicotine Dosages (mg/kg/day) During 1 of 3 Trimesters

	Trimester 1[a]			Trimester 2[b]			Trimester 3[c]		
	Nicotine			Nicotine			Nicotine		
	0.0	0.3	0.6	0.0	0.3	0.6	0.0	0.3	0.6
Mean Litter Size	8.5	6.0	10.0	8.0	10.0	6.5	4.5	9.0	4.0
Mean No Females/litter	2.5	2.5	3.5	5.5	4.5	3.0	1.5	5.5	2.0
Mean No Males/litter	6.0	3.5	6.5	2.5	5.5	3.5	3.0	3.5	2.0
Mean Litter Weight	68	71	66	59	66	70	83	61	82
Mean Female Weight	64	69	63	59	63	68	78	59	85
Mean Male Weight	69	73	68	61	69	72	85	65	81

Note: [a]Days 0 - 6. [b]Days 7 - 13. [c]Days 14 - 20.

Since the effects of food, water intake, and maternal weight gain during gestation may have influenced the number and weight of the pups in the litter at birth, these measures were used as covariates when differences in litter size and litter weight were evaluated. An interaction effect between gestation and dosage was found for litter size at birth, which could be attributed to a decrease in litter size over advancing gestation with saline and 0.6 mg/kg/day of nicotine, but a stable and slightly larger litter size with 0.3 mg/kg/day of nicotine over advancing gestation (F (4,9) = 3.88, $p < .05$). But this interaction effect disappeared when maternal fluid consumption and/or percent change in maternal body weight over gestation were controlled for by analysis of covariance. Therefore, the larger litter size at birth in animals treated with 0.3 mg/kg/day of nicotine during later trimesters can be explained by maternal body weight gain and fluid intake differences rather than a difference in prenatal treatment.

No differences were found among experimental groups in the number, and body weights of females and males at birth. No differences were found among the treatment groups in the male/female ratio in the litter at birth

or at weaning. This is contrary to Peters and Tang's (1982) finding that prenatal nicotine exposure reduced the number of male rats born and male birth weight. However, procedural differences in Peters and Tang's (1982) study may account for the discrepant findings. Peters and Tang administered nicotine (up to 6.0/mg/kg/day) in drinking water starting 6 weeks before mating and 40 mg/ml throughout the pregnancy. Therefore, the administration of nicotine prior to pregnancy, the higher chronic dosage used and/or oral rather than subcutaneous nicotine administration may result in female preference on the sex ratio of the offspring. Low (0.0-0.6 mg/kg/day) short term prenatal nicotine doses during any one of the 3 gestational trimesters do not alter male/female litter ratio or male/female birth weights.

Possible changes in maternal care or milk production on postnatal development were also controlled by weighing the offspring at birth and at regular intervals up to the time of weaning. Average body weight of males and females at birth and at regular 7 day intervals up to the time of weaning were not affected by either nicotine dosage or period of prenatal administration.

When either variable, litter size at weaning or average litter body weight at weaning, was used as a covariate with the other, a significant amount of the total variation was accounted for (67.16%) suggesting that these two variables are related. Therefore, litter size must be considered when evaluating body weight.

Maternal mortality was limited to one dam that had been administered 0.3 mg/kg/day of nicotine during the first trimester. This animal died shortly after a difficult delivery. Of the four stillbirths delivered, examination of two of the pups failed to reveal any obvious deformities of the brain, face or extremities; one pup had a facial deformity (elongation of the face and head and an unusual growth near the left nostril) and a blood clot was observed near the left nostril in the other pup.

An autopsy performed on the dam revealed three additional pups. One pup was decomposed at the rear end but its brain appeared normal. Another pup had no apparent abnormalities in the extremities but the brain was decomposing. The third pup had no obvious deformities; however, the head was visibly

underdeveloped with the placenta of another pup pressed deeply under its neck. This dam was replaced by a litter-mate female to restore the total size of the group to $\underline{n}$=2.

Stillbirths were also found in the group that had been treated with saline in the first trimester. Since all of the stillbirths that were observed in this group occurred in the same litter, and since this dam had an unusually large litter (14 pups), these stillbirths might be attributed to ontogenetic differences of the dam, or to complications arising from an unusually large litter, rather than to the stress imposed by subcutaneous saline injections.

Of the animals that had died postnatally, brain deformities were displayed in 2 pups that had been treated with saline during the first trimester and 2 pups that had been treated with 0.6 mg/kg/day of nicotine during the first trimester. Autopsy revealed indentation of the cerebrum and cerebral hemispheres, which may have occurred because of a collapse of the lateral ventricles in all 4 pups.

Malformations were also observed in 2 animals that had been treated during the second trimester. One animal which had been prenatally exposed to 0.3 mg/kg/day

during this trimester had an abnormally short tail, which is generally due to the mother's accidentally biting it off on the day of birth, during cleaning. The other animal exposed to 0.6 mg/kg/day during the same trimester was blind. Autopsy revealed no formation of eyes in this animal.

The severity and types of malformations observed were consistent with the developmental stage at the time of prenatal nicotine administration as predicted by Rodier (1976). The abnormalities observed in the first trimester were more severe (i.e., involved brain deformities) and resulted in death. Malformations observed in animals prenatally exposed during the second trimester did not result in death and were consistent with this period of organogenesis. No physical abnormalities could be detected in animals prenatally exposed to saline or nicotine during the third trimester.

Part II: Developmental Signs and Reflexes

Signs and Reflexes

The mean age of the appearance of developmental signs and reflexes are presented in Table 7.

Righting reflex. This reflex appeared at a

noticeably earlier age when nicotine or saline were administered during trimester 1, rather than trimester 3 (F (1,96) = 7.93, $p < .01$, χ^2 (1,96) = 6.95, $p < .01$).

Insert Table 7 about here

Palmar grasp. There was a difference in the appearance of palmar grasp due to gestation periods only (F (2,127) = 6.34, $p < .01$). This reflex was retarded for pups treated with nicotine in the second trimester as compared to the first trimester (F (1,99)=9.73, $p < .01$), or the third trimester (F (1,90)=8.72, $p < .01$). There was also a trend, irrespective of the gestational periods, for increasing nicotine dosage to result in an increasingly later appearance of the palmar grasp.

Since both palmar grasp and righting reflex involve coordination, it could be hypothesized that these two measures are related. However, dose and gestational effects on the development of these reflexes appear to be unrelated since when either variable is used as a covariate to control for the effects of the other

Table 7

Mean Age (Days) of the Appearance of Developmental Signs and Reflexes in Rats Treated with 1 of 3 Nicotine Dosages (mg/kg/day) During 1 of 3 Trimesters.

		Trimester 1[a]			Trimester 2[b]			Trimester 3[c]		
		Nicotine			Nicotine			Nicotine		
	n	0.0	0.3	0.6	0.0	0.3	0.6	0.0	0.3	0.6
Righting Reflex	145	1.33	1.16	1.21	1.73	1.15	1.75	1.44	1.70	1.38
Palmar Grasp	136	5.65	5.63	6.10	6.20	6.40	6.00	5.89	5.67	5.75
Pinna Detachment	145	2.56	2.47	3.00	2.00	2.00	2.00	2.00	2.10	2.38
Ear Opening	134	12.00	12.58	12.45	12.00	12.65	12.62	12.22	12.44	12.88
Primary Coat Devel.	140	4.11	3.68	4.62	4.40	3.65	3.69	5.00	3.95	4.38
Fur Devel.	135	8.06	8.53	8.52	8.00	9.00	8.54	8.56	9.44	8.38
Incisor Erup.	135	7.00	7.58	7.57	7.00	8.00	7.62	8.00	8.00	9.38
Cliff Avoidance	137	6.00	6.00	6.05	6.00	6.00	6.00	4.89	6.44	6.63
Eye Opening	133	14.88	15.11	14.85	16.00	15.10	15.00	14.78	14.94	14.63
Visual Placing	134	15.53	16.11	16.00	16.00	16.00	15.77	15.67	15.56	15.38

Note: [a]Days 0 - 6. [b]Days 7-13. [c]Days 14-20.

variable, little variance is accounted for.

Pinna Detachment. Pinna detachment occurred later with dose 3 (0.6 mg/kg/day) in both trimester 1 and trimester 3 whereas no dosage effects were detected in the second trimester. This differential effect of dosage and gestation led to an interaction between gestation and dosage (F (4, 136) = 4.16, $p < 0.1$). In addition, there were differences due to both gestation (F (2,136)=72.47, $p < .01$) and dosage (F (2,136) = 13.19, $p < .01$) alone. Pinna detachment appeared later in animals prenatally treated in the first trimester, than either trimester 2 (F (1,107) = 110.19, $p < .01$; χ^2 (1,107) = 54.54, $p < .01$), or trimester 3 (F (1,96) = 42.19, $p < .01$). These differences in the appearance of pinna detachment did not change when the effects of ear opening were controlled for by analysis of covariance.

Ear opening. Ear opening was delayed by increasing nicotine dosage but was not affected by gestation. Dose 1(0.0 mg/kg/day) resulted in an earlier appearance of ear opening than either dose 2 (0.3 mg/kg/day) (F (1,91) = 32.26, $p < .01$; χ^2 (1,91) = 24.08, $p < .01$), or dose 3 (0.6 mg/kg/day) (F (1,75) = 34.08, $p < .01$; χ^2 (1,75) = 22.75, $p < .01$). The control for pinna detachment by analysis of covariance did not affect this differential

dose effect.

Primary coat. The primary coat developed earlier with dose 2 than with dose 1, or dose 3 (F (1,93) = 14.76, $p < .01$; F (1,101) = 9.91, $p < .01$, respectively), across all 3 trimesters resulting in an interaction effect between gestation and dosage (F (4,131) = 3.56, $p < .01$). This dose effect was still present when development of fur was considered as a covariate (F (2,125) = 5.85, $p < .01$).

Fur development. Fur appeared earlier with saline in trimesters 1 and 2 but not in trimester 3. This differential effect resulted in an interaction between gestation and dosage (F (4,126) = 6.34, $p < .01$). There was also a trend for advancing gestation to result in a greater delay in the appearance of fur, particularly when comparing trimesters 1 and 3 (F (1,90) = 21.95, $p < .01$; χ^2 (1,90) = 15.78, $p < .01$). A dosage effect was also present. Dose 2 resulted in a greater overall delay of fur development than either dose 1 (F (1,91) = 60.79, $p < .01$), or dose 3 (F (1,97) = 19.87, $p < .01$), and dose 3 resulted in a greater delay than dose 1 (F (1,76) = 10.56, $p < .01$). When development of primary coat was used as a covariate, the above mentioned effects remained significant and were not related to development

of fur.

Incisor eruption. Incisor eruption was delayed with both advancing gestation and increasing dosage. Trimester 3 resulted in a more pronounced delay of incisor eruption appearances than either trimester 1 (F (1,190) = 25.59, $p < .01$), or trimester 2 (F (1,98) = 10.51, $p < .01$; χ^2 (1,98) = 8.26, $p < .01$). The dosage effect was due to a later appearance of incisor eruption with administration of dose 2 than dose 1 (F (1,91) = 17.11, $p < .01$) and of dose 3 than dose 1 (F (1,76) = 10.68, $p < .01$; χ^2 (1,76) = 15.62, $p < .01$).

Cliff Avoidance. Drug dose had similar effects when administered prenatally in trimester 1 or trimester 2, but higher doses of nicotine resulted in a greater delay of cliff avoidance in the third trimester only (all significant at $p < .01$). This differential effect of gestation and dosage led to an interaction between these two variables (F (4,128) = 25.72, $p < .01$). The additional variance explained by visual placing as a covariate (F (1,124) = 40.66, $p < .01$) suggests that cliff avoidance and visual placing are related. Eye opening as a covariate did not explain any additional variation, thus showing that appearance of cliff avoidance and eye opening are unrelated.

Eye opening. Age of eye opening decreased with higher doses of nicotine in trimester 2, whereas no such trends were evident in trimesters 1 or 3. This differential effect of drug dosage and gestation resulted in an interaction effect (F (4,124) = 4.38, $p < .01$). Eye opening was delayed in animals prenatally treated during trimester 2 when compared to trimester 1 (F (1,96) = 7.49, $p < .01$), and trimester 3 (F (1,89) = 9.49, $p < .01$). When visual placing was controlled for by analysis of covariance, 42% of the variance explained could be accounted for by this variable. Therefore, appearance of eye opening and visual placing appear to be related. Analysis of covariance suggests that eye opening and cliff avoidance are not related. Therefore, appearance of eye opening and cliff avoidance were found to be related to visual placing but not to each other.

Visual placing. There was a difference in the appearance of visual placing among gestation periods only (F (2,125) = 5.79, $p < .01$). Nicotine administration in trimester 3 resulted in an earlier appearance of visual placing as compared to trimester 1 (F (1,89) = 6.89, $p < .01$), or trimester 2 (F (1,76) = 10.25, $p < .01$). Cliff avoidance as a covariate accounted for 37% of the explained variance and caused a dosage effect to

emerge (F (2,124) = 7.36, $p < .01$).

Eye opening as a covariate also accounted for much of the explained variance (50.5%) but did not result in a significant dosage effect and decreased the significance level of the gestation effect from $p < .01$ to $p < .05$. When both the effects of eye opening and cliff avoidance were controlled by analysis of covariance, a gestation effect ($p < .01$), and a dosage effect ($p < .01$) were present. Refer to Table 8 for F-ratios of visual placing and its covariates.

Insert Table 8 about here

To control for possible differences inherent in the litters, all of the developmental measures were analyzed using mother of litter as a covariate. This variable did not explain any additional variation for either righting reflex or incisor eruption but did account for a significant amount of the variance ($p < .01$) for all of the other developmental measures. See Table 9 for F-ratios of developmental measures and covariates.

Table 8

F - Ratios and P - Values For Visual Placing and Covariates.

	Covariate	Gest	Dose	Gest X Dose
Visual Placing		5.79**	1.77	2.33
Visual Placing With Eye Opening as Covariate	22.44**	4.20*	2.81	2.22
Visual Placing With Cliff Avoinance As Covariate	23.06**	5.87**	7.36**	3.21*
Visual Placing With Eye Opening And Cliff Avoidance As Covariates	23.71**	4.52*	10.16**	1.91

Note: F - ratio significant *P < .05 and **P < .01.
d.f. for gestation and dosage (2,124); for interaction (4,124); and for covariates (1,124).

Insert Table 9 about here

Using litter differences as a covariate significantly increased the retarding effect of advancing gestation for cliff avoidance. Control for effects of litter reduced the gestation effect for development of the primary coat from $p < .01$ to $p < .05$, and also reduced the retarding effect of trimester 2 on eye opening (still significant at $p < .01$).

Litter differences as a covariate reduced the facilitative effect of higher doses for eye opening and increased the facilitative effects of higher doses for visual placing (from non-significance to $p < .01$).

The interaction effect between dosage and gestation was higher for ear opening and visual placing (from non-significance to $p < .01$) when litter effects were controlled. The interaction effect decreased for cliff avoidance, eye opening and palmar grasp (from $p < .05$ to non-significance).

In summary, the appearance of the physical signs and reflexes were differentially affected by the different nicotine doses and periods of prenatal drug

Table 9

F-Ratios and P-Values for Developmental Measures and Litter as Covariate

	COV	GEST	DOSE	GESTXDOSE
Righting Reflex		3.88*	0.99	2.19
Righting Reflex with Cov	1.80	3.78*	0.92	2.98*
Palmar Grasp		6.34**	0.33	2.20
Palmar Grasp with Cov	6.97**	8.54**	0.02	1.39*
Pinna Detachment		72.47**	13.69**	4.16**
Pinna Detachment with Cov	37.03**	99.03**	13.01**	12.30**
Ear Opening		0.90	17.69**	1.87
Ear Opening with Cov	9.56**	1.52	25.64**	6.37**
Primary Coat		4.77**	9.94**	3.56**
Primary Coat with Cov	13.13**	3.89*	9.85**	5.66**
Fur Development		14.52**	36.86**	6.34**
Fur Development with Cov	45.39**	28.94**	57.13**	13.10**
Incisor Erup		18.93**	11.26**	5.95**
Incisor Erup with Cov	0.10	20.31**	12.31**	7.44**
Cliff Avoidance		0.75	18.13**	25.72**
Cliff Avoidance with Cov	47.53**	3.65*	28.64**	24.15**

Table continues....

Table 9

	COV	GEST	DOSE	GESTXDOSE
Eye Opening		6.88**	3.13*	4.38**
Eye Opening with Cov	5.75**	5.80**	2.35	3.94**
Visual Placing		5.79**	1.77	2.33
Visual Placing With Cov	89.01**	22.52**	7.91**	16.51**

Note Cov = litter as covariate

F - ratio significant *p < .05 and **p < .01.

d.f. for gestation and dosage (2,124); for interaction (4,124) and for covariate (1,124).

administration.

Advancing gestation had a retarding effect on the appearance of righting reflex, fur development, incisor eruption and cliff avoidance whereas it had a facilitative effect on visual placing. Palmar grasp and eye opening were delayed with nicotine exposure in the second trimester only and cliff avoidance was delayed with exposure to nicotine in the third trimester only. Appearance of primary coat was not affected by trimester.

Higher nicotine doses retarded the appearance of incisor eruption, cliff avoidance and ear opening. Appearance of fur was more greatly retarded by 0.3 mg/kg/day than 0.6 mg/kg/day of nicotine. Pinna detachment appearance was retarded by the highest nicotine dose (0.6 mg/kg/day) only.

Righting reflex, eye opening and palmar grasp were not differentially affected by the different doses. The development of the primary coat was facilitated by nicotine (0.3 mg/kg/day), and the development of visual placing was facilitated by 0.6 mg/kg/day of nicotine.

Sexual Development

Body weight at sexual development. In females, as

gestation advanced, weight at vaginal opening increased with 0.6 mg/kg/day of nicotine but decreased with 0.3 mg/kg/day of nicotine. Whereas in males, as gestation advanced, weight at descent of testes increased with 0.6 mg/kg/day of nicotine, but decreased with 0.3 mg/kg/day during the second trimester only. See Table 10 for cell means. This differential effect of gestation, dosage and sex, led to an interaction effect among these 3 variables (F (4,131) = 4.58, $p < .01$).

Insert Table 10 about here

An interaction effect between gestation and dosage was also present due to the emergence of sexual development at a heavier weight with dose 3 but at a lighter weight with dose 2 as gestation advanced (F (4,131) = 5.09, $p < .01$). Weight differences at sexual development were also due to both gestational period (F (2,131) = 6.48, $p < .01$) and dosage (F (2,131) = 15.13, $p < .01$). Animals prenatally exposed to nicotine during trimester 3 weighed more at sexual development than

Table 10

Mean Weight (gm) at Sexual Development in Rats Prenatally Treated with 1 of 3 Nicotine Dosages (mg/kg/day) During 1 of 3 Trimesters.

	NICOTINE DOSAGE			
TRIMESTER	0.0	0.3	0.6	MEAN
FEMALES				
1[a]	125 (n=5)	124 (n=5)	115 (n=7)	121.3 (n=17)
2[b]	110 (n=11)	111 (n=9)	131 (n=5)	117.3 (n=25)
3[c]	136 (n=3)	108 (n=11)	155 (n=4)	133.0 (n=18)
MEAN	123.7 (n=19)	114.3 (n=25)	133.7 (n=16)	
MALES				
1[a]	181 (n=12)	181 (n=7)	189 (n=13)	183.7 (n=32)
2[b]	187 (n=5)	175 (n=11)	189 (n=7)	183.7 (n=23)
3[c]	195 (n=6)	183 (n=7)	197 (n=4)	191.7 (n=17)
MEAN	187.7 (n=23)	179.7 (n=25)	191.7 (n=24)	

Note: [a]Days 0 -6, [b]Days 7-13, [c]Days 14-20.

animals exposed to nicotine during trimester 1 (F (1,72) = 7.09, $p < .01$), or trimester 2 (F (1,71) = 11.07, $p < .01$). Dose 2 exposed animals were lighter than animals prenatally exposed to either saline (F (1,80) = 5.54, $p < .02$), or dose 3 (F (1,78) = 31.53, $p < .01$), and animals exposed to saline prenatally weighed less than animals exposed to dose 3 (F (1,70) = 7.57, $p < .01$). Dose 3 exposed animals were heaviest at sexual development.

Females were much lighter than males at sexual development (F (1,131) = 849.9, $p < .01$). These differences were not affected by litter, when used as a covariate.

Age at sexual development. Females developed sexually at an earlier age than males (F (1,131) = 322.7, $p < .01$). See Table 11 for cell means.

Insert Table 11 about here

Although animals prenatally exposed to dose 2 (0.3 mg/kg/day) were lighter at sexual development, females in this experimental group were the youngest, whereas

Table 11

Mean Age (Days) at Sexual Development in Rats Prenatally Treated with 1 of 3 Nicotine Dosages (mg/kg/day) During 1 of 3 Trimesters

	NICOTINE DOSAGE			
TRIMESTER	0.0	0.3	0.6	MEAN
	FEMALES			
1[a]	45.8 (n=5)	44.4 (n=5)	43.4 (n=7)	44.5 (n=17)
2[b]	44.1 (n=11)	44.4 (n=9)	47.8 (n=5)	45.4 (n=25)
3[c]	47.7 (n=3)	44.4 (n=11)	49.3 (n=4)	47.1 (n=18)
MEAN	45.9 (n=19)	44.4 (n=25)	46.8 (n=16)	
	MALES			
1[a]	52.8 (n=12)	55.1 (n=7)	53.2 (n=13)	53.7 (n=32)
2[b]	52.2 (n=5)	52.4 (n=11)	53.0 (n=7)	52.5 (n=23)
3[c]	53.0 (n=6)	53.6 (n=7)	52.5 (n=4)	53.0 (n=17)
MEAN	52.7 (n=23)	53.7 (n=25)	52.9 (n=24)	

Note: [a]Days 0-6, [b]Days 7-13, [c]Days 14-20.

males in this group were the oldest at appearance of sexual development.

All animals weighed more at sexual development when exposed to nicotine during the last trimester but females in this group were oldest at vaginal opening whereas males in the group were youngest at testes descent. Therefore, dose 2 appears to retard sexual development in males but facilitates sexual development in females. Conversely, treatment during the last trimester retarded sexual development in females whereas it facilitated sexual development in males.

Part III: Postnatal Behavioral Testing

The data from the behavioral testing was evaluated by analysis of variance and when appropriate, by analysis of covariance. No overall sex differences were found on any of the measures except for body weight. Data for all of the other measures were pooled for males and females for the repeated measures analysis. A total of 133 animals were tested in the open field and 132 animals were tested for avoidance learning.

Emergence From Home Cage

Mean emergence latency scores for the different

experimental groups are presented in Table 12.

Insert Table 12 about here

Differences in latency to emerge from home cage were found for both gestation and dosage. Animals prenatally exposed to dose 2 or dose 3 had shorter emergence latencies than saline treated animals (F (1,86) = 8.80, $p < .01$ and F (1,77) = 3.87, $p < .05$, respectively). Therefore, animals prenatally exposed to nicotine displayed more spontaneous movement in a familiar surrounding than animals prenatally treated with saline. The dose of nicotine administered prenatally was important in affecting spontaneous activity since the lower dose (0.3 mg/kg/day) of nicotine had the more pronounced effect. This finding is consistent with other findings that low doses of nicotine stimulate activity (Fitzgerald et al., 1985; Silvette et al., 1962).

The gestation effect was due to shorter emergence latencies in animals exposed to the drug in the second

Table 12

Mean Emergence From Home Cage Latencies (sec) For Rats Prenatally Treated with 1 of 3 Nicotine Dosages (mg/kg/day) During 1 of 3 Trimesters

	NICOTINE DOSAGE			
TRIMESTER	0.0	0.3	0.6	MEAN
1[a]	289.8 (n=17)	196.5 (n=12)	208.9 (n=20)	231.7 (n=49)
2[b]	220.7 (n=16)	159.7 (n=20)	188.0 (n=13)	189.5 (n=49)
3[c]	225.9 (n=9)	204.1 (n=18)	276.3 (n=8)	235.4 (n=35)
MEAN	245.5 (n=42)	186.8 (n=50)	224.4 (n=41)	

Note: [a]Days 0-6, [b]Days 7-13, [c]Days 14-20.

trimester than animals exposed to the drug in the first trimester (F (1,92) = 5.40, $p < .01$). Therefore, prenatal drug administration during the second trimester resulted in more active animals than drug administration in the first trimester.

Open Field Behavior

Differences in start box defecation scores and field defecation scores among the prenatal treatment groups disappeared when total defecation was controlled by analysis of covariance. Dose-gestation interaction differences in total defecations were also reduced but still significant at $p < .05$ when box defecation was used as a covariate. The control for arena defecations also decreased the level of significance for total defecation differences between gestation groups from $p < .003$ to $p < .018$ and between dosage groups from $p < .009$ to $p < .011$. See Table 13 for F-ratios of defecation measures and covariates.

Insert Table 13 about here

Table 13

F - Ratios and P - Values For Defecation Scores and Covariates

	Covariates	Gest	Dose	Gest X Dose
DEFT		6.05**	4.95**	3.25*
DEFT With DEFB As Covariate	796.7**	2.2	2.40	2.54*
DEFB		4.37*	4.71*	1.97
DEFB With DEFT As Covariate	743.2**	0.695	2.26	1.30
DEFA		2.34	2.46	2.56*
DEFA With DEFT As Covariate	37.35**	0.695	2.26	1.30
DEFT With DEFA As Covariate	41.84**	4.12*	4.64*	1.95

Note: DEFT = Defecation Total. DEFB = Defecation in box. DEFA = Defecation in arena. *F* - ratio significant **P* < .05 and ***p* < .01. d.f for gestation and dosage (2,124); for interaction (4,124) and for covariate (1,124).

This indicates that total defecation differences between the groups are more important than either arena or box defecation differences. Therefore, only the total defecation differences will be discussed.

The mean number of total defecations for the different experimental groups over the 4 days of open field testing are presented in Figure 1.

Insert Figure 1 about here

Total defecation scores were similar for the 3 dosage conditions during the first and second trimesters; but during the third trimester, the number of total defecations for the 4 days increased with doses 2 and 3 but not with saline. This differential effect resulted in an interaction between gestation and dosage ($F(4,124) = 3.25$, $p < .01$). This interaction effect disappeared when litter differences were controlled by analysis of covariance. A trend for late-trimester drug exposure to result in higher defecation scores was also present. In particular, animals prenatally treated in the third trimester defecated significantly more than

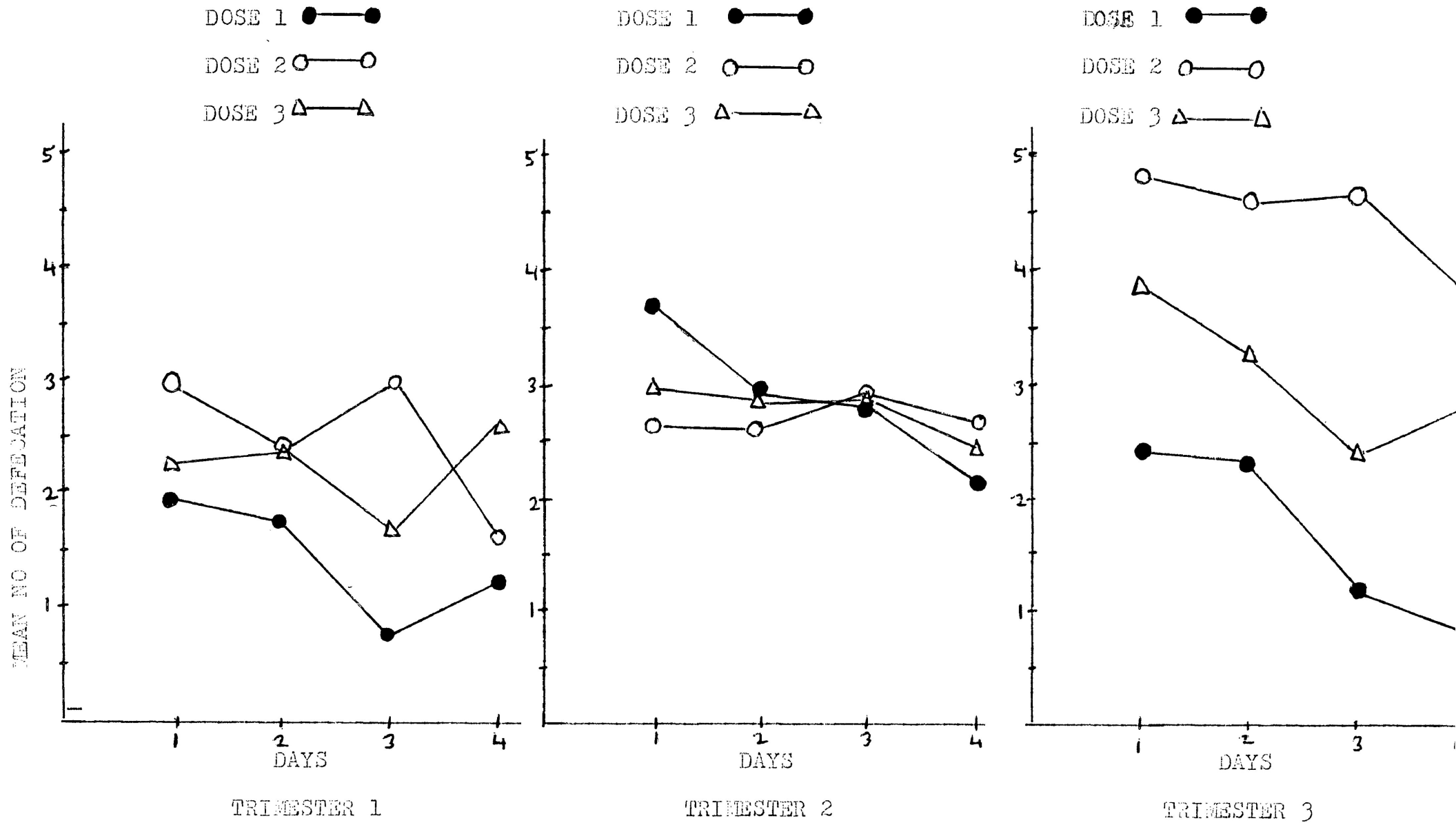

Figure 1. Mean total Defecation Score During 4 Daily 5-minute exposures to the open-field in rats prenatally treated with dose 1 (0.0 mg/kg/day) dose 2 (0.3 mg/kg/day) or dose 3 (0.6 mg/kg/day) of nicotine during 1 of 3 trimesters.

animals treated in the first trimester (F (1,72) = 7.35, p < .01). Dose also affected defecation scores. Animals prenatally exposed to dose 2 (0.3 mg/kg/day) defecated more than animals exposed to saline (F (1,80) = 9.06, p < .01).

Total defecation in the open field appears to be a sensitive index of prenatal nicotine effects on postnatal emotional reactivity. Animals prenatally treated with nicotine during the later trimesters were found to be more emotional as reflected by their higher defecation scores. Animals prenatally exposed to dose 2 (0.3 mg/kg/day) were also found to be more reactive as compared to animals prenatally exposed to dose 1 (0.0 mg/kg/day) or dose 3 (0.6 mg/kg/day). This was the same group that displayed shortest emergence latencies from a familiar surrounding.

Since several of the open field activity measures were found to be highly correlated (i.e., sections crossed, latency, number of entries, time out, and crossing of center squares), analysis of covariance was used to identify the more important measures. Refer to Table 14 for correlations between the open field measures.

Insert Table 14 about here

Table 15 gives the F-ratios for the open field measures and covariates.

Insert Table 15 about here

More variance could be accounted for by the variables of number of entries when either time out or latency were used as covariates than when the number of entries was controlled for by analysis of covariance for either time out or latency. Therefore, number of entries seems to be a more appropriate measure in explaining differences among experimental groups than either time out or latency to enter the open field.

Dosage differences in number of entries measure became non-significant and dose x gestation interaction differences increased to a $p < .05$ level of significance when sections crossed was used as a covariate. Whereas, when number of entries was controlled for by analysis of

Table 14

Correlation Coefficients Among Measures of Open Field

	1	2	3	4	5
(1) Sections Crossed					
(2) Latency	-.78**				
(3) Number of Entries	-.89**	-.87**			
(4) Crossing Center Squares	.81**	-.64**	.69**		
(5) Time Out	.91**	-.81**	.88**	.71**	

Note: Coefficients are statistically significant at

**$p < .01$ (n=133)

Table 15

F - Ratios and P - Values For Open Field Measures and Covariates

	Covariate	Gest	Dose	Gest x Dose
Sections Crossed (S)		2.69	7.24**	1.50
Latency (L)		1.98	2.28	1.10
Number of entries (N)		2.30	3.85*	1.38
Crossing center squares (C)		2.39	5.13**	2.88*
Time out (T)		0.45	3.63*	0.85
S with L as covariate	238.78**	0.82	6.28**	3.04*
S with N as covariate	611.8**	3.05	4.55*	3.13*
S with C as covariate	262.32**	0.59	2.87	2.36
S with T as covariate	725.22**	6.20**	5.08**	1.77
L with S as covariate	217.51**	0.14	1.22	2.62*
L with N as covariate	425.09**	0.99	0.32	2.73
N with S as covariate	5 73.74**	2.64	1.17	3.00*
N with L as covariate	4 37.56**	1.30	1.89	3.01*
N with T as covariate	500.48**	5.54**	0.34	3.03*
C with S as covariate	262.32**	0.20	0.07	3.76*
T with S as covariate	656.41**	3.77*	1.61	1.14
T with N as covariate	483.08**	3.48*	0.18	2.47*

Note: F ratio significant *$p < .05$ and **$p < .01$. d.f. for gestation and dosage (2,124), for interaction (4,124) and for covariates (1,124).

covariance for the sections crossed measure, dosage differences were reduced to a $p < .05$ level of significance but dosage x gestation effect increased to $p < .05$ and more of the total variation could be accounted for by the variables. Sections crossed, therefore, seems to be the more appropriate measure in explaining prenatal differences. Since the same amount of total variation can be accounted for when either sections cross or center squares is used as a covariate with the other, only sections crossed will be discussed.

The mean number of sections crossed over the 4 testing days are presented in Figure 2.

Insert Figure 2 about here

A dosage effect was present in the number of sections crossed over the 4 days of open field testing (F (2,124) = 7.24, $p < .01$). Animals prenatally treated with dose 2 (0.3 mg/kg/day) were more active than animals prenatally treated with dose 1 (F (1,90) = 11.88, $p < .01$) or dose 3 (F (1,87) = 5.66, $p < .02$). This

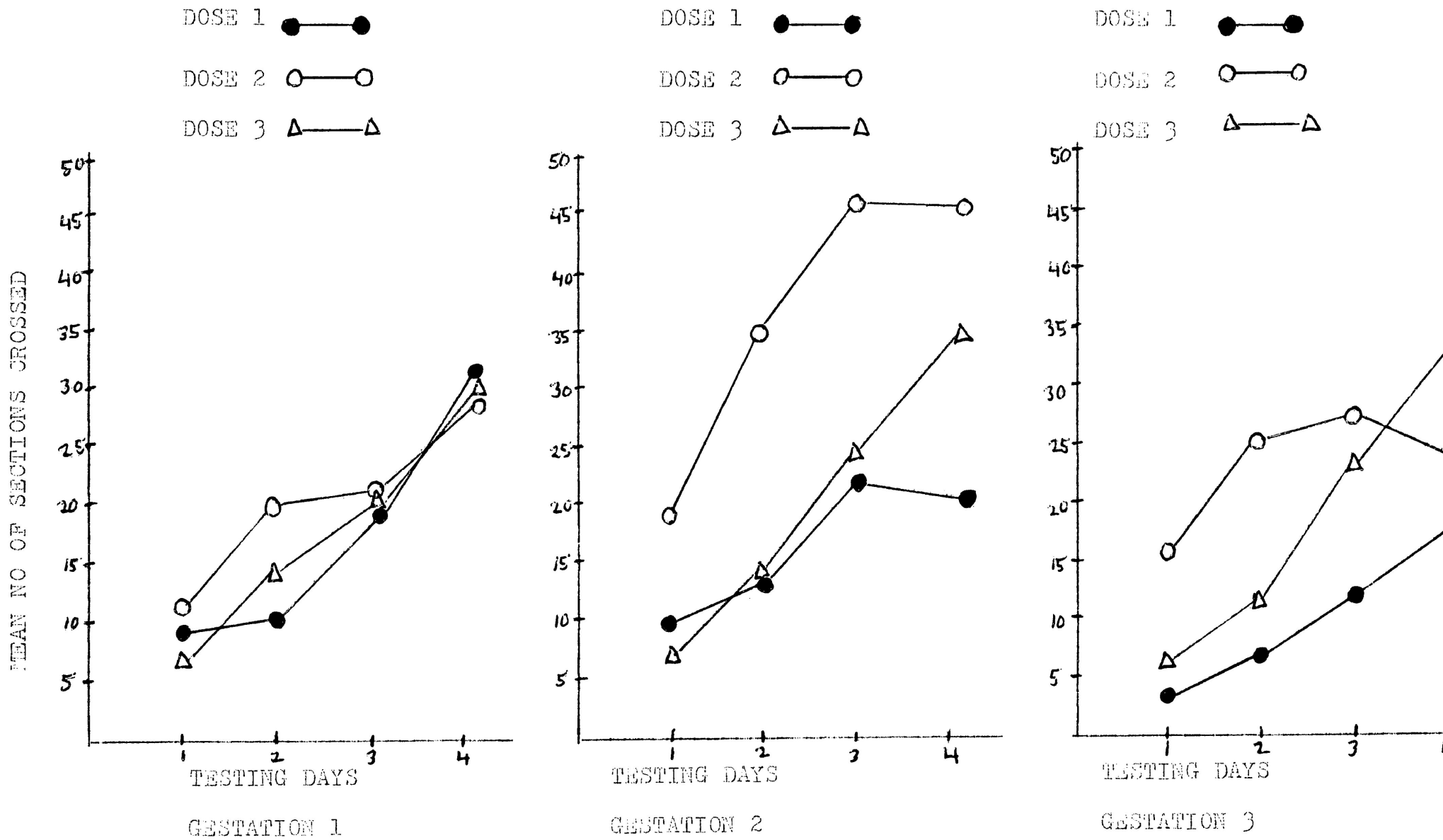

Figure 2. Mean total sections crossed during 4 daily 5-minute exposures to the open-field in rats prenatally treated with dose 1 (0.0 mg/kg/day), dose 2 (0.3 mg/kg/day) or dose 3 (0.6 mg/kg/day) of nicotine during 1 of 3 trimesters.

higher activity level of dose 2 exposed animals is consistent with their activity level in a familiar surrounding as reflected by shorter emergence from home cage.

An overall progressive increase in the number of sections crossed over the 4 days of testing (F (3,396 = 6.71, $p < .01$) was apparent. This change over days was significant at $p < .01$ for all groups except animals prenatally exposed to saline during trimesters 2 and 3 (only significant at $p < .05$). When litter effects were controlled for by analysis of covariance, the significance level of the dosage effect increased and a gestation effect, which can be attributed to the higher activity level of trimester 2 animals, emerged (F (2,123) = 4.74, $p < .01$). This is the same trimester in which activity in a familiar surrounding was highest as evidenced by shorter emergence from home cage latencies.

These results show that the stage of development at the time of prenatal nicotine administration and the dosage level are both important factors in determining the effects of prenatally administered nicotine on open field activity.

Unconditioned Escape Response

Unconditioned Escape Response (UER) scores were determined to ensure equal motivation for each animal for the avoidance learning task. See Table 16 for mean scores of UER values.

Insert Table 16 about here

There was a tendency for animals prenatally treated with higher doses of nicotine to require less intense foot shock to reach criteria. In particular, animals prenatally treated with dose 3 were more sensitive to electric shock than animals prenatally treated with saline (F (1,71) = 5.23, $p < .05$). This difference in sensitivity to footshock could suggest an alteration in the motivation level which may reflect an increased

Table 16

Mean Scores (ma) For Unconditioned Escape Responses of Rats Prenatally Exposed to 1 of 3 Nicotine dosages (mg/kg/day) During 1 of 3 Trimesters

	NICOTINE DOSAGE			
TRIMESTER	0.0	0.3	0.6	MEAN
1[a]	.36 (n=17)	.38 (n=12)	.33 (n=20	.36 (n=49)
2[b]	.38 (n=16)	.35 (n=20)	.33 (n=13)	.35 (n=49)
3[c]	.39 (n=9)	.37 (n=18)	.39 (n=8)	.38 (n=35)
MEAN	.38 (n=42)	.37 (n=50)	.35 (n=41)	

Note: [a]Days 0-6, [b]Days 7-13, [c]Days 14-20.

level of arousal in this dosage group. However, this dosage difference disappeared when litter differences was used as a covariate in the analysis. Therefore, the dosage differences observed can be accounted for by differences inherent in the litters rather than prenatal treatment. Further research is needed to see whether dosage effects of UER's will emerge when a larger number of litters are used.

Gestation did not affect UER sensitivity although there was a trend for increasing gestation to result in higher UER scores.

Either-Way Avoidance Training

The mean number of avoidance responses during avoidance training is presented in Figure 3.

Insert Figure 3 about here

Gestational periods had an affect on avoidance acquisition over the 4 days of training (F (2,123) = 3.57, $p < .05$). In particular, animals prenatally treated with nicotine during the second trimester consistently showed higher levels of avoidance than animals

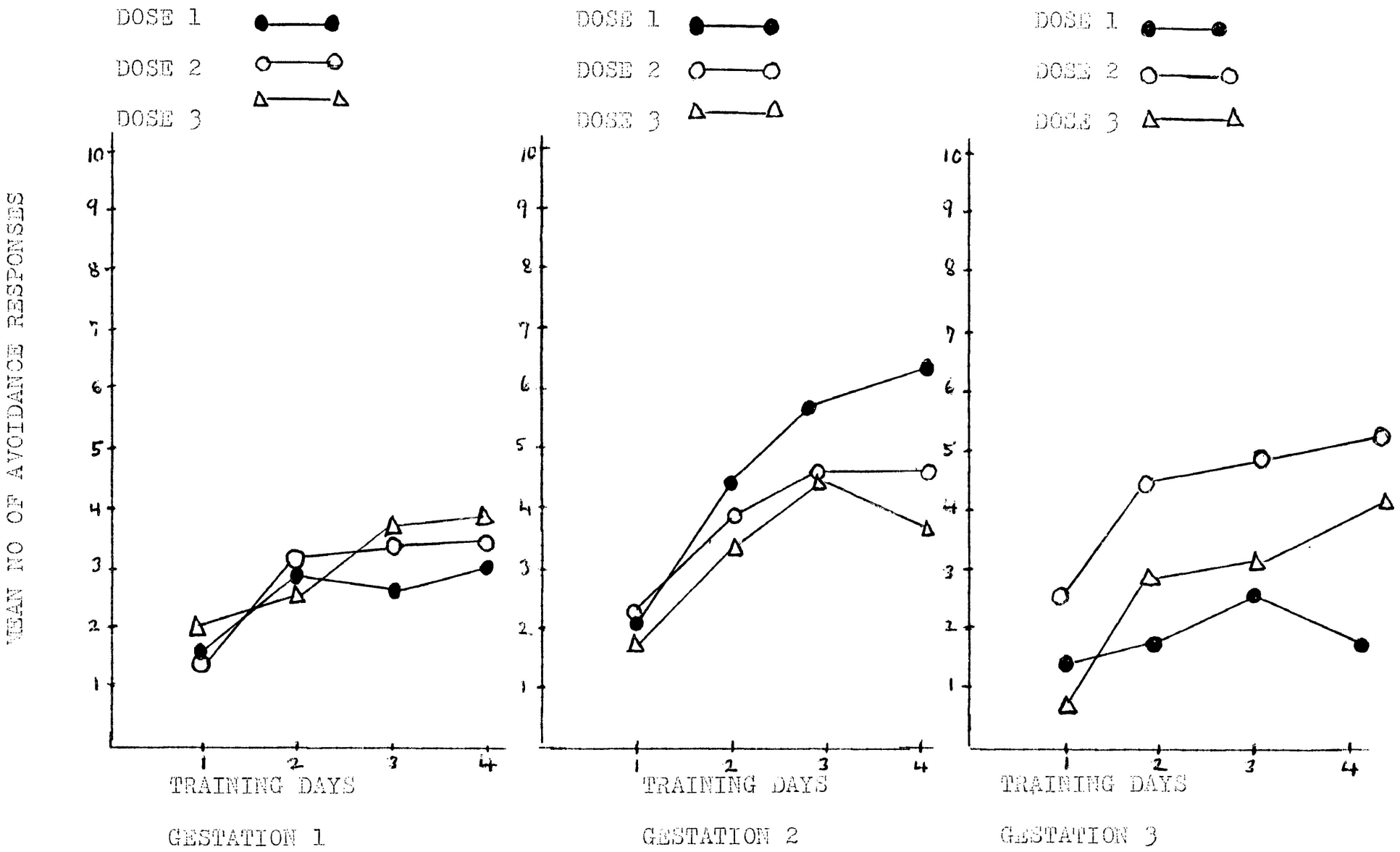

Figure 3. Mean number of avoidance responses during 4 days on either-way avoidance training in rats prenatally treated with dose 1 (0.0 mg/kg/day), dose 2(0.3 mg/kg/day) or dose 3 (0.6 mg/kg/day) of nicotine during 1 of 3 trimesters.

prenatally treated with nicotine during the first trimester ((F (1,85) = 7.22, p < .01). Since this gestation effect decreased when total number of no escapes was used as a covariate, this variable can explain some of the differences in avoidance acquisition between the gestational groups.

When litter differences were controlled by analysis of covariance, the gestation effect disappeared. Therefore, differences in overall avoidance acquisition between trimester 1 and trimester 2 groups can be attributed to differences between litters rather than to prenatal treatment.

All groups showed improved avoidance acquisition over the 4 days of training (F (3,393) = 46.89, p < .01) except animals prenatally exposed to saline during the first trimester (F (3,48) = 2.72, p > .055), and during the third trimester (F (3,24) = 1.03, p > 0.4).

To determine the effects that response complexity may have on avoidance learning, data for two-way and one-way avoidance responses were extrapolated from either-way scores. See Table 17 and Table 18 for mean 1-way and 2-way responses, respectively.

The two-way avoidance response has previously been demonstrated to be more complex than the one-way

avoidance response, when either direction is permitted (Satinder, 1977). But in the present study, no overall preference for either one-way or two-way avoidance responses were found.

Insert Table 17 about here

Insert Table 18 about here

The finding that animals did not differentiate between one-way or two-way responses contradicts Persson's (1984) finding that SHS rats, whether prenatally exposed to saline, or 0.3 mg/kg/day of nicotine, had a preference for one-way responses.

It is possible that the discrepancy between the two studies in the difference in direction of avoidances made by this line is due to procedural differences in the duration of prenatal nicotine exposure, or this difference may have been due to the large genetic

Table 17

Mean Number of 1-way Avoidance Responses During the Days of Avoidance Training in Rats Prenatally Exposed to 1 of 3 Nicotine Doses During 1 of 3 Trimesters (day 0-6, day 7-13, or day 14-20).

	DAY			
NICOTINE DOSE	1	2	3	4
	GESTATION 1			
0.0 mg/kg/day	0.5	1.6	1.5	1.5
0.3 mg/kg/day	0.5	1.4	2.0	2.1
0.6 mg/kg/day	0.9	1.4	1.9	2.1
	GESTATION 2			
0.0 mg/kg/day	0.9	2.7	3.1	3.1
0.3 mg/kg/day	1.0	2.2	2.7	2.3
o.6 mg/kg/day	0.6	1.7	2.2	1.6
	GESTATION 3			
0.0 mg/kg/day	0.7	0.7	1.4	0.8
0.3 mg/kg/day	0.9	0.5	2.9	2.1
0.6 mg/kg/day	0.3	2.1	1.9	2.3

Table 18

Mean Number of 2-way Avoidance Responses During the 4 Days of Avoidance Training in Rats Prenatally Exposed to 1 of 3 Nicotine Doses During 1 of 3 Trimesters (day 0-6, day 7-13, or day 14-20).

	DAY			
NICOTINE DOSE	1	2	3	4
	GESTATION 1			
0.0 mg/kg/day	0.9	1.3	1.2	1.5
0.3 mg/kg/day	0.8	1.7	1.3	1.2
0.6 mg/kg/day	0.9	1.1	1.6	1.8
	GESTATION 2			
0.0 mg/kg/day	1.1	1.6	2.3	3.0
0.3 mg/kg/day	1.2	1.7	1.8	2.2
0.6 mg/kg/day	1.3	1.7	2.3	2.3
	GESTATION 3			
0.0 mg/kg/day	0.6	0.9	1.0	1.1
0.3 mg/kg/day	1.4	2.9	2.3	3.3
0.6 mg/kg/day	0.4	0.8	1.1	1.9

differences present within the SHS rat.

The number of one-way avoidance responses tended to increase over the 4 days of training (F (3,393) = 30.83, $p < .01$). Only animals treated with saline during trimester 3 failed to show a difference in the number of one-way responses over the 4 days of avoidance training.

The total number of two-way avoidance responses across the 4 days of avoidance training increased with prenatal exposure to dose 2 as gestation period advanced, but decreased for animals prenatally treated with dose 1 and dose 3 during the last trimester. This differential effect of gestation and dosage led to an interaction effect (F (4,123) = 2,84, $p < .05$).

An overall increase in the number of two-way avoidance responses over days (F (3,393) = 13.87, $p < .01$) was also present, but only animals prenatally exposed to saline during trimesters 2 and animals treated with dose 2 during the third trimester showed a significant increase in the number of 2-way responses over days (F (3,45) = 3.47, $p < 0.05$ and F (3,51) = 4.76, $p < 0.05$, respectively).

Avoidance Performance Under the Effects of Nicotine

The mean number of avoidance responses during postnatal challenge with nicotine is presented in Figure 4.

Insert Figure 4 about here

There was an overall difference in avoidance between animals prenatally treated with nicotine during the first trimester and the second trimester on all 4 days of postnatal nicotine challenge. Animals prenatally exposed during trimester 2 consistently avoided at a higher rate than animals prenatally exposed during trimester 1 with all 4 postnatal nicotine doses (0.0, 0.2, 0.3, and 0.4 mg/kg/day) regardless of the order in which the dosages were given (F (1,85) = 8.46, $p < 0.01$). This difference was also found for 2-way avoidance performance with postnatal nicotine (F (1,85) = 6.18, $p < .05$), and is consistent with the gestation effect found during training; i.e., animals prenatally exposed during the second trimester consistently showed higher levels of avoidance than animals treated during trimester 1. But this difference in avoidance responding with postnatal nicotine between trimester 1 and 2 disappeared, as it did during avoidance training, when

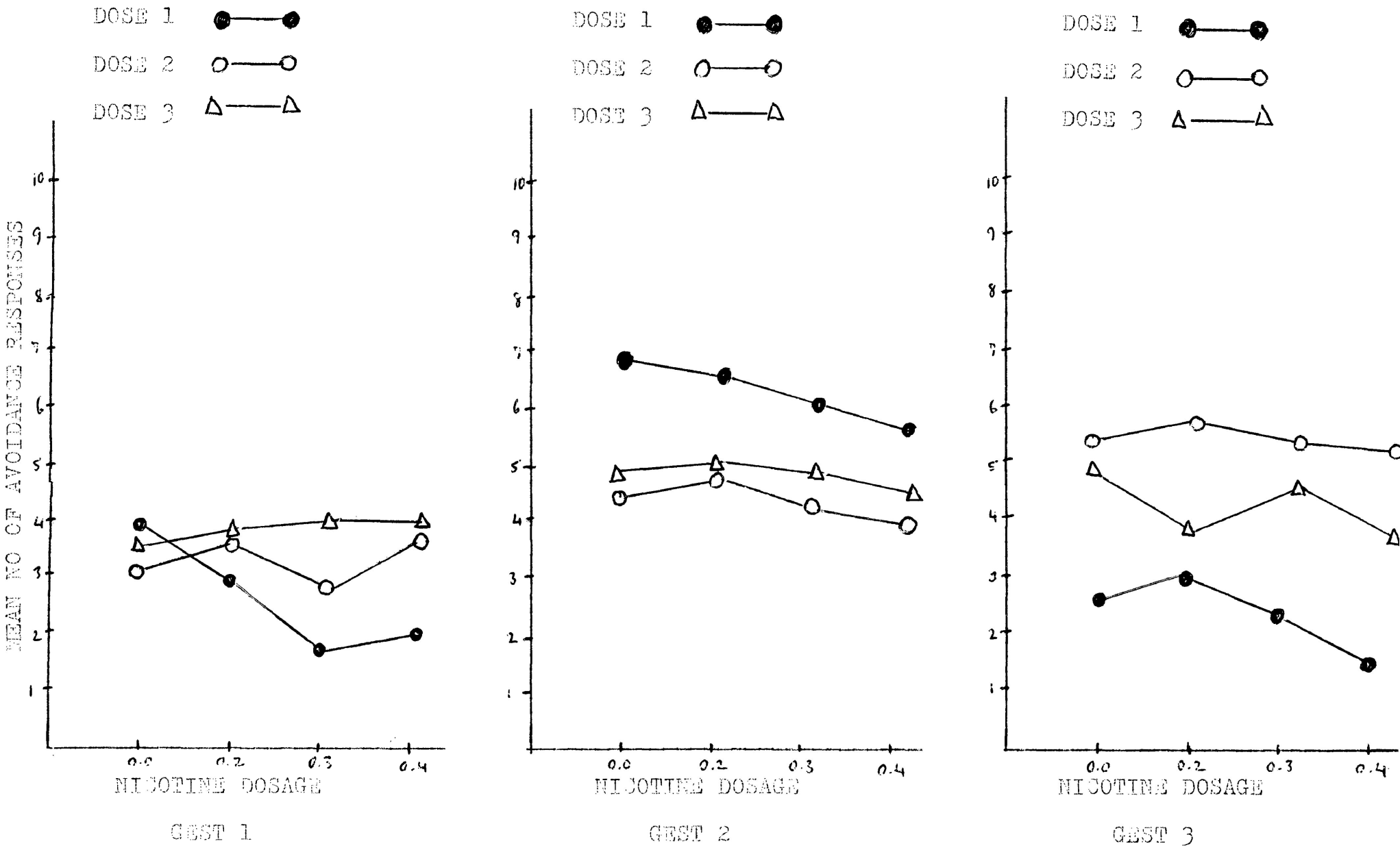

Figure 4. Effect of postnatal injection of 4 doses of nicotine on established avoidance behavior in rats prenatally treated with 0.0 mg/kg/day, 0.3 mg/kg/day or 0.6 mg/kg/day of nicotine during 1 of 3 trimesters.

litter effects were controlled by analysis of covariance.

A dose-response was observed for number of avoidances in animals prenatally treated with saline during the first trimester (F (3,48) = 4.96, $p < .01$). This effect could be attributed to a decrease in the number of avoidances as postnatal nicotine dose increased up to 0.3 and a slight increase in avoidance with 0.4 mg/kg nicotine postnatally. No other treatment groups exhibited differences in avoidance performance with different nicotine doses.

During the postnatal challenge with nicotine, no overall preference for one-way or two-way avoidance responding was found. Response preference was not affected by prenatal nicotine dose or gestational period and did not change with the different postnatal nicotine doses. See Table 19 and Table 20 for mean one-way and two-way avoidance responses under postnatal challenge with nicotine respectively.

Insert Table 19 about here

Table 19

Mean Number of One-Way Avoidance Responses With Post-Natal Injection of 4 Doses of Nicotine in Rats Prenatally Exposed to 1 of 3 Nicotine Doses During 1 of 3 Trimesters (Days 0-6, Days 7-13, or Days 14-20).

	Postnatal Nicotine (mg/kg)			
Prenatal Nicotine (mg/kg/day)	0.0	0.2	0.3	0.4
	GESTATION 1			
0.0	2.3	1.1	0.8	0.9
0.3	2.0	1.9	2.1	2.2
0.6	1.5	1.7	1.4	1.8
	GESTATION 2			
0.0	3.1	3.3	3.6	2.9
0.3	2.1	2.0	2.1	1.7
0.6	1.8	1.9	1.8	1.9
	GESTATION 3			
0.0	1.3	1.3	1.2	1.1
0.3	1.8	3.1	2.8	2.0
0.6	2.4	1.8	1.8	1.4

Insert Table 20 about here

No dose response effects were found for the number of two-way avoidances in any of the treatment groups but dose response effects were found in the number of one-way avoidances for animals prenatally treated with saline during trimester 1 and for animals prenatally exposed to 0.3 mg/kg/day of nicotine during the third trimester.

Animals prenatally treated with saline during trimester 1 displayed increasingly lower one-way avoidance performance with increasing postnatal nicotine doses with a slight improvement with postnatal 0.4 mg/kg of nicotine (F (3,48) = 4.41, $p < .01$). This is the same trend observed for this group's overall avoidance performance with postnatal nicotine.

Animals prenatally treated with 0.3 mg/kg/day during the third trimester performed poorest with postnatal saline and best with postnatal dose 2 (0.2 mg/kg/nicotine), and less well with the higher nicotine doses (F (3,51) = 2.90, $p < .05$). It is interesting to

Table 20

Mean Number of Two-Way Avoidance Responses With Post-Natal Injection of 4 Doses of Nicotine in Rats Prenatally Exposed to 1 of 3 Nicotine Doses During 1 of 3 Trimesters (Days 0-6, Days 7-13, or Days 14-20).

	Postnatal Nicotine (mg/kg)			
Prenatal Nicotine (mg/kg/day)	0.0	0.2	0.3	0.4
	GESTATION 1			
0.0	1.6	1.8	1.0	1.2
0.3	1.1	1.8	0.9	1.7
0.6	2.1	2.1	2.8	2.3
	GESTATION 2			
0.0	3.8	3.6	2.5	3.0
0.3	2.2	2.9	2.2	2.4
0.6	3.1	3.0	2.8	2.3
	GESTATION 3			
0.0	1.2	1.6	1.1	0.7
0.3	3.4	2.7	2.6	3.3
0.6	2.5	2.1	3.0	2.5

note that for both of these groups displaying differential postnatal nicotine effects, avoidance performance was best under the postnatal dose most comparable to the dose of nicotine received prenatally. Therefore, animals prenatally exposed to saline during the first trimester performed best under postnatal saline and animals prenatally treated with 0.3 mg/kg of nicotine (0.1 mg/kg administered in the morning and 0.2 mg/kg administered in the afternoon) performed best with 0.2 mg/kg of postnatal nicotine. Therefore, for both of these experimental groups, a drug dependent state exists. It is not known why this dose effect was not found in the other experimental groups.

Body Weight

With advancing gestation, overall body weights during either open field testing or during avoidance testing decreased with dose 2, increased with dose 3 and followed a V-shaped curve (lower during the second trimester) with dose 1. This differential effect of dosage over gestation resulted in a significant interaction effect between dosage and gestation ($p < .01$ for all measures). Differences in body weights were also found due to prenatal dose effects only. Animals

prenatally exposed to 0.3 mg/kg/day weighed less than animals exposed to saline or 0.6 mg/kg/day (significant at $p < .01$ for all measures) both during open field and during avoidance testing. However, no overall differences in average body weight at 7 day intervals could be attributed to either gestation or dosage when the data was analyzed by litter. See Table 21 for mean weights of the experimental groups at 7-day intervals.

Insert Table 21 about here

General Discussion

The results of the present study demonstrate that both nicotine dosage and period of nicotine administration during prenatal development can differentially affect the development and behavior of rats.

Since maternal food consumption and fluid intake and percent weight gain over gestation were not affected by the different prenatal conditions, developmental and behavioral differences observed between groups cannot be

Table 21

Mean Body Weight of Litters on Day 1, 7, 14, 21, 28, 35, and 42 of Rats Prenatally Treated with 1 of 3 Nicotine Doses (mg/kg/day) During 1 of 3 Trimesters (day 0 -6, day 7 - 13 or day 14 -20)

Dose	Day 1	7	14	21	28	35	42
	Gestation 1						
0.0	5.7	13.8	25.2	39.7	67.9	96.6	125.5
0.3	6.7	14.4	25.7	40.5	70.8	97.3	125.7
0.6	6.4	13.2	23.8	38.3	65.9	95.5	124.5
	Gestation 2						
0.0	6.1	12.8	24.6	36.1	59.5	88.2	111.6
0.3	6.7	13.9	26.1	39.1	66.0	90.9	118.3
0.6	6.4	15.5	29.4	44.2	69.5	97.1	125.7
	Gestation 3						
0.0	6.4	17.5	33.7	52.6	83.2	107.9	138.0
0.3	6.3	12.7	22.7	35.0	61.3	85.3	109.7
0.6	6.8	16.9	32.9	51.3	82.2	109.6	139.4

attributed to nutritional differences between dams during gestation. Furthermore, failure to find adverse effects on litter size at birth or at weaning, litter weight, male-female ratio or postnatal mortality lend support that the intended non-morphological levels of nicotine were used. Therefore, the minimal doses employed in the present study should have detected periods of greatest susceptibility, and not produced the less clear effects evidenced by higher doses, which caused behavioral deficits to appear on days other than those of greatest vulnerability.

The results show that delays in the appearance of developmental signs and reflexes are specific to the time of prenatal nicotine administration as well as to the dosage level used.

Since rat fetal brain starts to differentiate and develop during the second trimester (Rodier, 1976), prenatally administered nicotine may affect or delay the prenatal and/or postnatal development of the CNS primarily through the second and third trimester, and especially in high doses. Later, as a consequence of this intervention, a delay in the appearance of developmental signs or reflexes may result. Visual placing was the only sign to appear latest in the first

trimester. In general, nicotine's effects were probably limited during this stage of pregnancy since the brain has not yet differentiated. Thus, action during this trimester is probably indirect. Possible mechanisms whereby nicotine could exert indirect effects include impairment of uterine and/or blood circulation and transfer, essential for fetal growth and development (Abel, 1980), which in turn may influence brain development.

Nicotine readily crosses the placenta in all animals and accumulates in fetal lung, adrenals, kidney, and brain (Abel, 1980), and reduced DNA and protein levels have been suggested (Barnes et al., 1981). Therefore, effects of nicotine during the second and third trimester are probably direct and may cause developmental delays which persist in the postnatal period. The general finding in the present study that developmental signs and reflexes were delayed in the later trimesters, support such direct effects.

Al-Hachim and Mahmood (1985) also show greater vulnerability of later trimesters in delaying CNS maturation and development as demonstrated through audiogenic seizures. They found that prenatal nicotine prolonged the latency, and delayed the onset and

extinction of audiogenic seizures. These effects were more pronounced in the third trimester. The finding that palmar grasp and eye opening were delayed with nicotine exposure in the second trimester implicate this trimester as more susceptible to the development of these signs. Since eye opening is needed for sight, and one animal treated during this trimester failed to form eyes, this trimester appears to be more crucial in the development of sight.

The finding that higher nicotine doses caused a greater delay in the appearance of physical signs and reflexes is consistent with Al-Hachim and Mahmood's (1985) finding of greater adverse effects on CNS development and maturation with higher nicotine doses.

In the present study it was found that 0.3 mg/kg/day of nicotine administered prenatally retards sexual development in males whereas administration of nicotine in the third trimester retards sexual development in females. This sex difference in susceptibility to prenatal treatment becomes meaningful when considering that age is more crucial in the sexual maturation of female rats whereas body weight is more crucial in the sexual maturation of male rats (Satinder, 1984).

Since 0.3 mg/kg/day of nicotine results in lower weighted animals, and weight is crucial for male sexual development, 0.3 mg/kg/day of prenatal nicotine specifically delays sexual maturation in males. Since trimester 3 animals are the heaviest, but females in this group are oldest and age is more crucial for sexual development in females, trimester 3 has specific detrimental effects on the appearance of sexual development in females.

Therefore, prenatal treatment which has the effect of reducing weight (0.3 mg/kg/day), will more likely retard sexual development in males whereas, prenatal treatment which results in heavier animals but older females will retard sexual development in females.

The higher activity levels in both a familiar and unfamiliar surrounding and the greater fearfulness (higher defecation scores) exhibited by animals prenatally exposed to 0.3 mg/kg/day of nicotine when compared to saline exposed animals and the higher activity levels of groups exposed to drug during the second trimester (as reflected by shorter emergence latencies and more sections crossed in the open field) suggest that the effects of nicotine administration in utero cannot be generalized since both the time of drug

administration and dosage of nicotine used are crucial in determining nicotine's effect on postnatal emotional reactivity.

An optimal dose of prenatal nicotine is necessary for increased postnatal activity since the higher dose (0.6/mg/kg/day) failed to increase postnatal emotional reactivity levels. The greater effect of 0.3 mg/kg/day of nicotine may be explained on the basis of an inverted U-shaped arousal curve. As nicotine levels increase, physiological arousal increases, but when levels are too high (0.6 mg/kg/day), the animal is overaroused and interference of activity occurs.

The higher activity levels found in prenatal groups exposed to 0.3 mg/kg/day of nicotine or to the drug during the second trimester are inconsistent with previous prenatal studies. Mice prenatally exposed to tobacco smoke displayed depressed open field activity (Baer, McClearn and Wilson, 1980). An important variable in previous prenatal nicotine or tobacco smoke studies was the quantity of the nicotine or smoke administered. Since Baer et al.'s (1980) study found increased perinatal mortality rate in animals exposed to smoke, the level of nicotine in the smoke reaching the embryo appears to be higher than the 0.3 mg/kg/day dose

employed in the present study. Alternatively, other tobacco components or hypoxia could have been responsible for the effects found. Other procedural differences which may account for the discrepant findings include smoke administration throughout the entire pregnancy rather than for short term periods or the possible differential effects of nicotine on mice rather than rats.

Contrary to the present findings, Martin and Becker (1970) failed to find any change in activity when nicotine administration was limited to the gestational period but did find an increase in activity when animals were exposed to nicotine during gestation and nursing. But, the observed higher activity levels in animals prenatally administered 0.3 mg/kg/day of nicotine in the study, is consistent with postnatal nicotine effects. Spontaneous locomotor activity is stimulated by low doses (0.2 mg/kg subcutaneously) of nicotine, whereas larger doses have a depressing effect on activity (Fitzgerald, Oettinger, and Bättig, 1985). It is possible that the shorter nicotine exposures during gestation had a more pronounced effect on postnatal activity than continuous prenatal nicotine treatment during the entire gestation and that this effect

mimicked postnatal administration effects. As previously described, maternal metabolic changes causing decreases in dosage to the developing organism that may be operating in prolonged prenatal nicotine exposure would be avoided by employing short term treatment periods (Wilson, 1975). This would explain the apparent discrepancy between the present study's finding of increased open field activity and Martin and Becker's (1971) failure to find an increase in activity in rats exposed to nicotine throughout the pregnancy.

The present findings suggest that brief exposure to nicotine during different gestational trimesters does not affect subsequent either-way avoidance acquisition in adulthood. When litter differences were controlled, no specific trimester in which animals were particularly susceptible to nicotine's effects were obtained. Failure to obtain a significant dosage effect on avoidance acquisition is consistent with previous findings involving chronic prenatal and postnatal nicotine administration. Martin and Becker (1971) found no effect of prenatal nicotine on reinforcement schedules and simple discrimination performance. Fleming and Broadhurst (1975) also found no effect of nicotine in doses slightly lower (0.016 to 0.25 mg/kg of nicotine

expressed as base) to those used in this experiment on two-way escape-avoidance conditioning in the RHA and RLA rats. Therefore, in low doses, nicotine does not appear to affect postnatal either-way avoidance learning.

But a more precise analysis of the effects of prenatal exposure to nicotine is permitted when examining different components of the either-way avoidance response. Since the either-way avoidance performance procedure allows identification of response choice to be made, this procedure quantifies several behavioral characteristics in one behavioral measure and permits analysis of the behavioral teratogenic effects of avoidance response complexity in more than one manner.

Trimester 2 appears to have produced animals capable of the more complex 2-way avoidance response as evidenced by increased number of 2-way avoidance responses in these groups over days. These prenatal groups appear to be superior to the other experimental groups since 2-way avoidances have been shown to be more complex than 1-way avoidances (Satinder, 1977). Since no significant differences in sensitivity to UER were found among groups, this difference in the more complex avoidance responding cannot be attributed to differences

in shock sensitivity. The superior performance of these groups appear to be attributed to their higher level of physiological arousal as evidenced by higher activity in the open field and shorter emergence from home cage latencies. With an increase in baseline arousal, effective avoidance response in a complex mode was enhanced.

In conclusion, the present findings show that the effects of nicotine are dependent on both dosage and time of nicotine administration. But identification of specific critical periods are precluded until further investigations are made. Due to practical constraints, the present study did not include an untouched group to control for effects of saline or stress involved in injection. The assumption that saline injection would not adversely affect development and behavior must be experimentally tested. Inclusion of groups administered nicotine throughout the pregnancy would have been informative. Such groups could provide information about whether short-term exposure maximizes or minimizes nicotine's effects and whether such effects are additive. More precise effects of nicotine on development and behavior may be obtained by a finer division of both the period of gestation in which

nicotine is administered and the prenatal dosage used. The present findings should also be replicated since behavioral effects are usually subtle and such replication would provide greater support for the effects found. Replication would also help validate the behavioral protocol employed in the present study.

Statistical control of litter differences proved to significantly affect many of the results in the present study. Further research could directly control for this variable by including several litters per group or examine genetic differences by transplanting embryos of half the litters between two pregnant females before nicotine administration.

The main contribution of the present study is that it is the first to examine the effects of different doses of nicotine during different trimesters on a variety of developmental and behavioral measures in the same experiment and in the same animals. From the present findings, it seems clear that consideration of nicotine dosage, developmental stage of the organism at the time of nicotine administration, and the interdependence of these variables are crucial in studying behavioral teratogenic effects of nicotine. Similar considerations should be employed in other prenatal behavioral teratogenic research.

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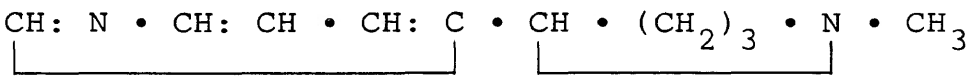
Appendix A

Nicotine Composition

Molecular Formula:



MW : 162.24

Minimum assay (acidimetric): 98%

Wt per ml at 20° C : 1.005 to 1.015 g

Refractive Index : 1.527 to 1.529

Manufactured by;

British Drug House Chemicals Limited,

Poole, England

Appendix B

Physical Signs and Their Approximate Appearance in Rats

(Alder and Zbinden, 1977)

Sign	Day of Appearance
Pinna Detachment	Day 2
Primary Coat of Downy Hair	Day 5
Incisor Eruption	Day 8
Development of fur	Day 9
Ear Opening	Day 11
Eye Opening	Day 14
Testes Descent	Day 25
Vaginal Opening	Day 30

Appendix C

Development of Reflexes and Their Time of Appearance in Rats

(Smart and Dobbing, 1971)

Test	Time of Appearance
Righting Reflex (1)	Day 1.8
Cliff Avoidance (2)	Day 4.8
Palmar Grasp (3)	Day 6.3
Visual Placing (4)	Day 17.6

(1) Rat placed on back turns over on ventral side

(2) Rat placed on edge moves away from end of table

(3) Grasps a paper clip if stroked with clip on forepaws

(4) Rat held by tail with vibrissae not touching surface lifts head and extends forelegs in direction of surface.