The Effects of Ethanol and Nicotine on the Brain Development of the Embryonic Chick: a Model for Substance Abuse During Pregnancy

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Table of Contents

I. Abstract

The chick embryo was used as an animal model to test the effects of ethanol and nicotine on the brain of the developing embryo. The experimental groups received 2 μl doses of either Howard Ringers solution, 0.5% ethanol, 5.0% nicotine, or a combined treatment of ethanol and nicotine at their respective doses. All treatments were given on days 1-7 of incubation, which is analogous to the first trimester of human gestation. Data was collected for the control, nicotine treated, and ethanol + nicotine treated groups. A moderately significant difference was found between the brain-to-body weight ratios of the control and ethanol + nicotine treated groups ($p < 0.05$). Significant differences were found between the control and nicotine treated groups for mean neuron counts of two cortical layers and the pyramidal thalamic cells ($p < 0.01$). Significant differences were also found between the control and ethanol + nicotine treated groups for all four neuronal counts taken ($p < 0.01$). The nicotine treated and ethanol + nicotine treated groups differed significantly for the mean neuron counts for both types of thalamic cells. These results suggest that nicotine treatments inhibit neuroblast division, which results in reduced cell density and brain-to-body weight ratios. The effects were more pronounced for the ethanol + nicotine treated group, suggesting the damage is compounded by the addition of ethanol.

II. Introduction

It is a widely accepted fact that maternal abuse of nicotine and alcohol has detrimental effects on the growing fetus. Concern with smoking during pregnancy began in the mid 1930s (Campbell, 1935; Campbell, 1936; Sontag and Wallace, 1935), gaining attention in 1957 when the conclusion was reached that smokers had twice the number of premature births than non-smokers (Simpson, 1957). In 1985, the harmful effects of maternal smoking on the fetus were termed "Fetal Tobacco Syndrome" (Nieburg et al., 1985). Severe fetal damage due to alcohol exposure had previously been described as Fetal Alcohol Syndrome (FAS) (Jones et al., 1973). It has been estimated that the likelihood of depressed fetal growth in utero is affected 2.4-times by alcohol use, 1.8-times by smoking, and 3.9-times by the combined use of the two substances (Sokol, 1981). However, it remains unknown what amount of alcohol or nicotine, if any, is acceptable to the fetus (Edwards, 1983).

Diminished fetal growth is related to both maternal smoking, which excites the central nervous system, and drinking, which depresses neuronal firing. Recent studies suggest that birthweight, which is an effective means to measure the "success of pregnancy" (Fried and O'Connell, 1987), is lowered only in pregnancies where the mother drinks 120g (1 drink=12g alcohol) or more of alcohol per week (Sulaiman et al., 1988). Another study concluded that low birthweight only occurred when alcohol intake was combined with smoking (Brooke et al., 1989). A study by Olsen et al. (1991) states that birthweight decreases as smoking and drinking increase (an inverse correlation). The negative effects on birthweight associated with alcohol were more pronounced in smokers than in non-smokers, suggesting an additive effect of alcohol and nicotine. This study by Olsen et al. also found more than a 500g birthweight difference between the two

extremes of alcohol and tobacco intake. A criticism of this study, and many like it, is that the data are collected through self-reports, which may result in underestimating substance abuse (Olsen et al., 1991). Nonetheless, these results are in agreement with Mills et al. (1984) who found that non-smokers with low to moderate drinking habits had little effect on the birthweight of their offspring. However, heavy smoking lowered the range of alcohol in which fetal effects were seen to 30-59g ethanol per week.

It has been shown that maternal smoking not only lowers birthweight but also increases the likelihood of fetal and perinatal death; there is also some evidence suggesting a relationship between smoking during pregnancy and congenital defects (Landesman-Dwyer and Emanuel, 1979). In a study concerned with the timing of maternal substance abuse, birthweight was affected by cigarette smoking in both the first and third trimester, with a more significant decrease associated with third trimester use (181.01g vs. 134.39g). In the same study, the ponderal index (PI), which is equivalent to weight x 100/length^3, was used to measure low birthweights that were accountable to problems in the third trimester. Heavy smoking throughout pregnancy and during the third trimester only showed lower ponderal indices. First trimester smoking only did not produce this effect. The low PI infants retained normal weight and length measurements by one year of age, suggesting a problem with in utero nourishment (Fried and O'Connell, 1987).

In a study in which pregnant rats were treated with alcohol and nicotine until gestational day 12 of a 20 day gestational period, no significant decreases in birthweight were found (Persaud, 1982). In another study, Peterson et al. exposed mice to both ethanol and tobacco smoke on gestational days 6 to 16. This resulted in significantly lower birthweight, length, and more fetal resorptions (1981). A study in which pregnant rats were given one combined treatment on day 9 yielded no significant changes (Lindenschmidt and Persaud, 1980). This

illustrates that combined prolonged exposure to both alcohol and nicotine is important in causing fetal damage.

A study using Fisher and Buffalo rats resulted in a 29% sterility increase in nicotine treated rats and a 4%-13.1% sterility increase in alcohol treated rats, as compared to controls (Riesenfeld and Oliva, 1987). The life spans of the rats were shortened in both cases. Correlations between this study and the case of maternal smoking include spontaneous abortion, decreased fertility, decreased birthweight of offspring, and a more difficult time conceiving. The latter two effects have been reversed in some cases where smoking was stopped prior to conception. In a further study in which the risk of spontaneous abortion was compared on a logistic scale, odds ratios rose by 1.26 for each drink per day and 1.20 for each half pack of cigarettes per day (Armstrong et al., 1992). These findings suggest a dose dependent effect of alcohol and nicotine on the developing fetus.

Maternal smoking during pregnancy is associated with lowered weight, height, and head circumference of the baby at birth (Barr et al., 1984). Smoking may also be responsible for a minor long term depression in growth. Smoking and alcohol intake have been found to have both singular and additive influences on birthweight. When infants were examined at eight months of age, those whose mothers drank and smoked heavily during pregnancy had the lowest weights, while those who were exposed to only one of the substances had higher weights. At birth, the nicotine effects were more prevalent than alcohol effects. However, at eight months, the effects of alcohol were more evident and the nicotine effects had begun to fade (Barr et al., 1984). Another study examined the conditions of infants at thirteen months who were exposed to "social" levels of alcohol and nicotine prenatally (Gusella and Fried, 1984). Moderate drinking during pregnancy has been shown to result in decreased birthweight, lowered performance on

motor and mental tests, and a higher incidence of tumors, while FAS is associated with mental retardation, facial disfiguration, depressed growth, and depressed central nervous system functioning. At thirteen months, the infants whose mothers drank socially scored lower on the mental developmental index (MDI) and on verbal tests, including speaking and understanding words. The mothers who smoked before pregnancy had offspring who scored lower on the psychomotor developmental index (PDI) and on tests of fine motor skills. Smoking during pregnancy resulted in lowered verbal comprehension performance. These studies suggest long term effects of prenatal alcohol and nicotine exposure and even effects from smoking before pregnancy (Barr et al., 1984; Gusella and Fried, 1984).

Many studies suggest that social background is related to substance abuse. In one study, it was suggested that Black and Hispanic women had less of a tendency to use a substance than White women (Abma and Mott, 1991). Additionally it was found that if the father and mother lived together, less substance abuse occurred. Younger, less-educated women smoked cigarettes more, while older, more-educated women were more inclined to drink alcohol. Prenatal care was more likely to be substandard if the social situation was unfavorable. Thus, it is important for these women to seek prenatal care to become informed about their potentially harmful behavior. Proper prenatal care and subsequent education about maternal substance abuse is critical as shown by evidence that the combined use of substances has a synergistic effect (Marbury et al., 1983; Powell-Griner and Rogers, 1987; Tennes and Blackard, 1980).

According to interviews of expectant mothers, those who smoke are twice as likely to drink than those who do not (Rind, 1991). Pregnant women with college educations were more likely to drink than those with a lesser education (30% vs. 21%). Older (35-40 years old) and nonmarried women also had a higher tendency to drink. It has also been shown that women are

more likely to quit drinking than smoking during pregnancy, but that mixed or hostile feelings about the pregnancy may influence unhealthy maternal behavior. In such cases, the addiction to alcohol or nicotine overrides maternal concern for the health of the fetus (Condon and Hilton, 1988).

It has been documented that only 20% of expectant mothers stop smoking during pregnancy, regardless of the accompanying higher risk of premature membrane rupture, placenta previa (faulty positioning of the placenta), and abruptio placentae (separation of the placenta from the uterine wall prior to the birth of the baby) (Prager et al., 1984). It has been found that smoking raises the risk of placenta previa and sudden infant death syndrome (SIDS) in White babies 2.6- and 2.2-fold, respectively (Feng, 1993). Smoking is thought to be harmful because of the nicotine and carbon monoxide content in cigarettes. A pregnant subject with a carboxyhemoglobin level of 10% (equivalent to smoking two packs a day) has been shown to raise the fetal level of carboxyhemoglobin by 10%-15%. This level equates to fetal blood flow being lowered by 60% (Longo, 1977). In sheep, nicotine injections have resulted in increased resistance of the uterine vascular system and reduced blood flow in the uterus. Catecholamine released into the bloodstream is thought to regulate these occurrences (Resnik et al., 1979). It has also been shown that maternal smoking slows the heart rate and breathing movements of the fetus (Lehtovirta et al., 1983).

Both alcohol and nicotine are able to traverse the placental barrier (Luck and Nau, 1984; Zorzano and Herrera, 1989). It is known that less alcohol is required to produce alterations in the brain than in other parts of the body (Zajac and Abel, 1992). It has also been found that the fetus is at higher risk for damage from alcohol and nicotine than from diabetes, Rh incompatibility, and other problems that are screened for during pregnancy (Sokol, 1981).

This experiment attempted to ascertain the damage caused by alcohol and nicotine exposure during the first trimester on subsequent brain development in the embryonic chick. The degree of microcephaly suffered by the chick embryos was gauged by measuring brain weight. After dissection, sections of the brain were prepared histologically and examined using light microscopy. Estimates of cell density in the embryonic brains were made by counting the number of nuclei in a specific area on a microscopic field. The brains affected by the alcohol and/or nicotine treatments had decreased brain-to-body weight ratios. Cell density determinations provided clues as to the cause of the decreased brain weights. Decreased cell density was indicative of cell growth. Thus, decreased brain weight accompanied by higher than normal cell density suggested that lack of cell growth is the cause of the low brain weight. In addition, the histology provided an additional method for determining cell abnormalities in the brains. The chick embryo was used as an animal model in this study because of developmental similarities to the human embryo. The chorioallantoic membrane (CAM) surrounding the chick embryo is homologous to the mammalian placenta. Alcohol and nicotine are able to pass through the CAM without being metabolized just as they cross the placental barrier during human gestation.

The results of the experiment may be applied to humans because the three week gestational period of the chick is analogous to the three trimester gestation of the human. It has been noted that brains develop according to a proportional time frame in all vertebrates (West, 1990). Much is known about the detrimental effects of alcohol and nicotine on the developing fetus. However, more studies are critical in order to educate the public about prenatal risks and how they may be avoided.

This study sought to determine the effects of early ethanol and nicotine use during a pregnancy in which the mother responds to prenatal advice and terminates smoking and drinking

after 13 weeks of pregnancy. This study specifically addressed the questions: (1). Are the combined effects of nicotine and ethanol additive or synergistic; (2). Is microencephaly due to the lack of neuroblast growth, and (3). Are major histological structures impeded in development as a result of the experimental treatments

III. Materials and Methods

Incubation

Fertile chicken eggs were supplied by Carolina Biological Supply Company. The regulations for care stated in "Carolina Fertile Chick Eggs Handling Instructions" were followed. The eggs were kept in an incubator maintained at 38.5°C and 80% humidity.

Treatments

The eggs were divided into four groups. The first group of eggs acted as the control group and received 2 µl doses of Howard Ringers solution on the first seven days of incubation. The second group received 2 µl doses of 5.0% nicotine in Howard Ringers solution on days 1-7. This mimicked the situation in which the mother smokes chronically during the first trimester of pregnancy. The third group of eggs were treated with a 2 µl dose of 0.5% ethanol in Howard Ringers solution on days 1-7. This treatment corresponded to "chronic" alcohol drinking by the expectant mother. The fourth set of eggs received a 2 µl combined treatment of ethanol and nicotine at their respective concentrations in Howard Ringers solution. This protocol mimicked the situation present when pregnant women abuse both nicotine and alcohol regularly during the first trimester. It is known that ethanol is able to cross the placental barrier. However, incomplete development and function of the fetal liver and hepatic enzymes result in the ethanol being metabolized at only half the rate of the mother (Rosell, 1974). Therefore, there is a possibility of the fetus having a higher blood alcohol content than the mother due to the reduced metabolic rate. For this reason, an upper limit of a nonlethal pharmacological dose of ethanol was chosen for the treatments in this study. An appropriate nicotine concentration was chosen by performing the following calculations. One cigarette contains 1.4 mg of nicotine. If the average smoker smokes ten cigarettes per day and 1% of the nicotine crosses the placental barrier, then

the fetus is exposed to 140 μg of nicotine per day. The average body weights of an unmanipulated chick and a human baby are 18.3 g (Gorman, 1992) and 3175.2 g, respectively. The ratio of average chick-to-human weight was multiplied by 140 μg to give approximately 1.00 μg as the upper limit for nicotine dosage per day. One-tenth of this amount (0.1 μg) was chosen in order to prevent the application of lethal doses to the chick embryos. Table 1 shows the treatments given to the chick embryos in each of the four groups.

After locating the embryo on the first day of incubation using a shadow illumination technique (candling), the shell was punctured in order to release the air sac and lower the embryo's position. A second hole or window was made in the shell above the location of the embryo and covered with scotch tape to prevent contamination. A Hamilton syringe was used to apply the different solutions directly to the chorioallantoic membrane (CAM). All preparations and treatments were done in a laminar flow hood. Sterile techniques were maintained by treating the syringes with 70% ethanol and using a separate syringe for each treatment group.

Body and Brain Weight Measurements

All of the chicks were removed from their shells and sacrificed on the sixteenth day. Any dead chicks were excluded from the remainder of the study. The live chicks were decapitated and the entire embryo was weighed on an analytical balance to the nearest hundredth of a gram. The heads were fixed in a solution of 30% sucrose (w/v) and 10% formalin (v/v) in water until the brains could be dissected out at a later date. Once the brains were removed from the skulls, they were weighed and fixed in the same manner mentioned above. The mean body weights, brain weights, and brain-to-body weight ratios are recorded in Table 2.

Histological Analysis

Seven brains were selected for histological analysis (three nicotine treated, three ethanol +

nicotine treated, and one control). Each brain was removed from the original fixative and placed in a fixative solution containing 20g sucrose per 100ml formalin.

The brains were cut into coronal sections at -17°C using a cryostat. The width of the cut was kept constant at 40 μm. The coronal sections were placed on glass slides and allowed to air-dry overnight. The slides were then dehydrated in 70% alcohol for 24 hours. The sections were subsequently stained with cresyl violet, rinsed with various alcohols, formaldehyde, butanol, and Hemo De, and coverslipped. Neuronal counts were taken from cortical layers IV and V at the pallial wall adjacent to the lateral ventricles and a comparison was done between the number of granular versus pyramidal cells in the thalamic area. A magnification of 312.5X was used for the neuronal counts. Ten separate counts were done of each area and the values averaged. An ocular etched with a circular area of 1964 μm^2 was used to perform the counts.

Statistical Analysis

Statistical analyses were performed using Statistical Tutor by Allen and Pittenger of the University of Georgia and Marietta College.

IV. Results

The control, nicotine treated, and ethanol + nicotine treated groups were the only groups to yield viable data due to high mortality in the ethanol treated group. Only one embryo was obtained from the ethanol treated group. The body weight of the embryo was 3.43g. The brain weight was not determined as the brain was not intact upon dissection. There were unusually high mortality rates in all four groups that was not attributable to the treatment received. This may be stated with confidence because the control group had the second highest mortality rate. For the remaining three groups, brain-to-body weight ratios were chosen as the most valid measure of brain growth because general body size differences were factored out by this method.

Table 3 shows the mean brain-to-body weight ratios for the three treatment groups and the number of chick embryos included in each group. A t-test showed no significant differences between the control and nicotine treated groups or the nicotine and ethanol + nicotine treated groups. A moderately significant difference was found between the ratios of the control and ethanol + nicotine treated groups.

The mean neuron counts observed within 1964 μm^2 areas of cortical layer IV and the number of cell counts performed for the three treatment groups are found in Table 4. A t-test showed significant differences between the control and nicotine treated and the control and ethanol + nicotine treated groups. No significant difference was found between the nicotine and ethanol + nicotine treated groups.

Table 5 shows the mean neuron counts taken from 1964 μm^2 areas of cortical layer V and the number of cell counts performed for the three treatment groups. A t-test showed significant differences between the control and nicotine treated and control and ethanol + nicotine treated groups. There was no significant difference between the nicotine and ethanol + nicotine treated

groups.

Table 6 records the mean neuron counts (granular cells) observed within 1964 μm^2 areas of the thalamic area and the number of cell counts performed for the three treatment groups. A t-test showed no significant difference between the control and nicotine treated groups. Significant differences were found between the control and ethanol + nicotine treated and nicotine and ethanol + nicotine treated groups.

The mean neuron counts (pyramidal cells) taken from 1963.5 μm^2 areas of the thalamic area and the number of cell counts performed for the three treatment groups. A t-test showed significant differences between the control and nicotine treated, control and ethanol + nicotine treated groups, and the nicotine and ethanol + nicotine treated groups.

These results indicate that the nicotine and ethanol + nicotine treated embryos do exhibit more brain damage than the chick embryos in the control group. The mean neuron count of the nicotine treated group differed significantly from that of the control group in both cortical layers. The number of pyramidal cells in the thalamic area of the nicotine treated brain also differed significantly from the brain of the control embryo. A moderately significant difference was found between the brain-to-body weight ratios of the chick embryos in the control and ethanol + nicotine treated groups. The mean neuron counts of the ethanol + nicotine treated group differed significantly from the control group for all counts taken and from the nicotine treated group for the granular and pyramidal cells of the thalamic area. No major histological structures showed signs of impeded development or other pathology.

Treatment Group	Treatment (days 1-7)
Control	2 µl of Howard Ringers solution
Ethanol	2 µl of 0.5% ethanol in Ringers
Nicotine	2 µl of 5.0% nicotine in Ringers
Ethanol + Nicotine	2 µl of 0.5% ethanol + 5.0% nicotine in Ringers

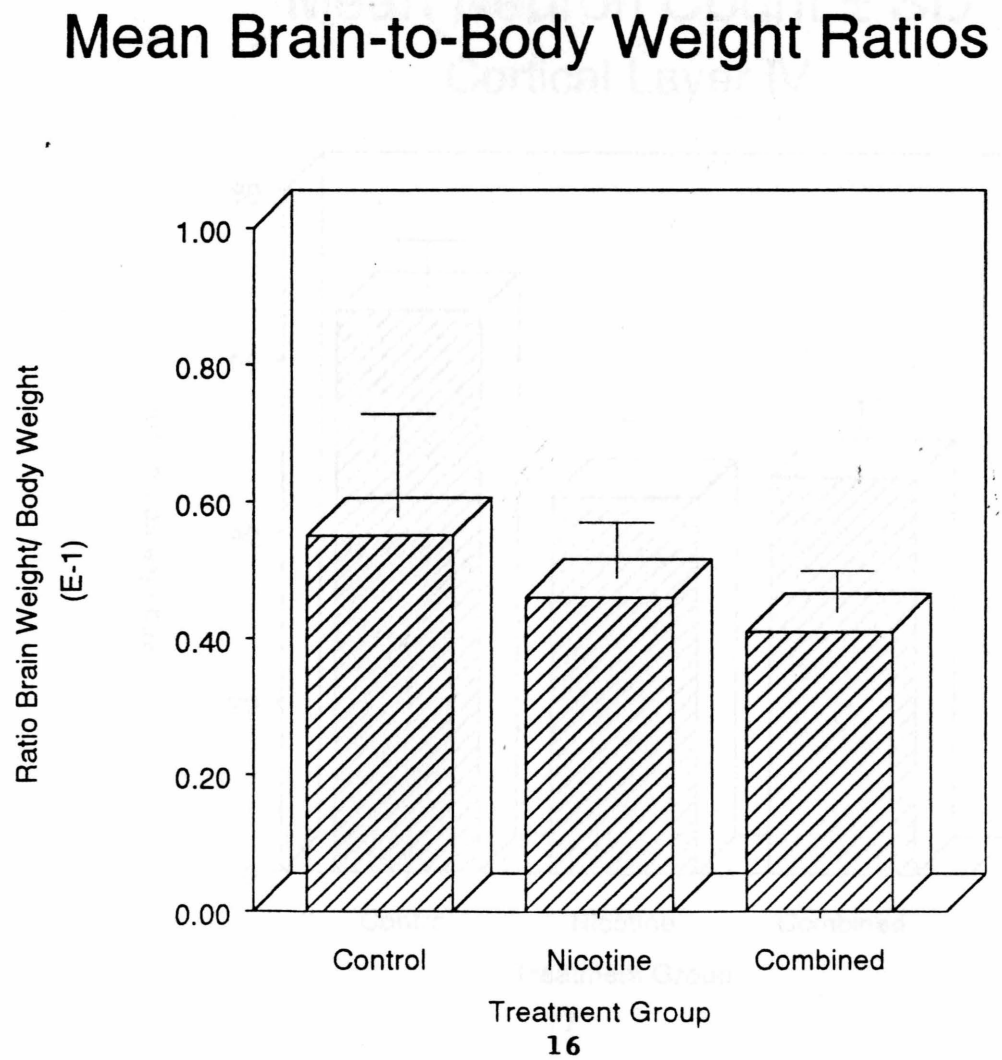
Table 1: The four different treatment groups and their respective treatments given on days 1-7 of incubation.

Treatment Group	Mean Body Weight (g) ± SD	Mean Brain Weight (g) ± SD	Mean Brain-to-Body Weight Ratio ± SD
Control	4.803 ± 2.329	0.242 ± 0.047	0.055 ± 0.015
Nicotine	7.705 ± 2.255	0.353 ± 0.090	0.046 ± 0.008
Ethanol + Nicotine	7.634 ± 2.556	0.299 ± 0.076	0.041 ± 0.006

Table 2: Mean body weights, brain weights, and brain-to-body weight ratios for each of the three treatment groups.

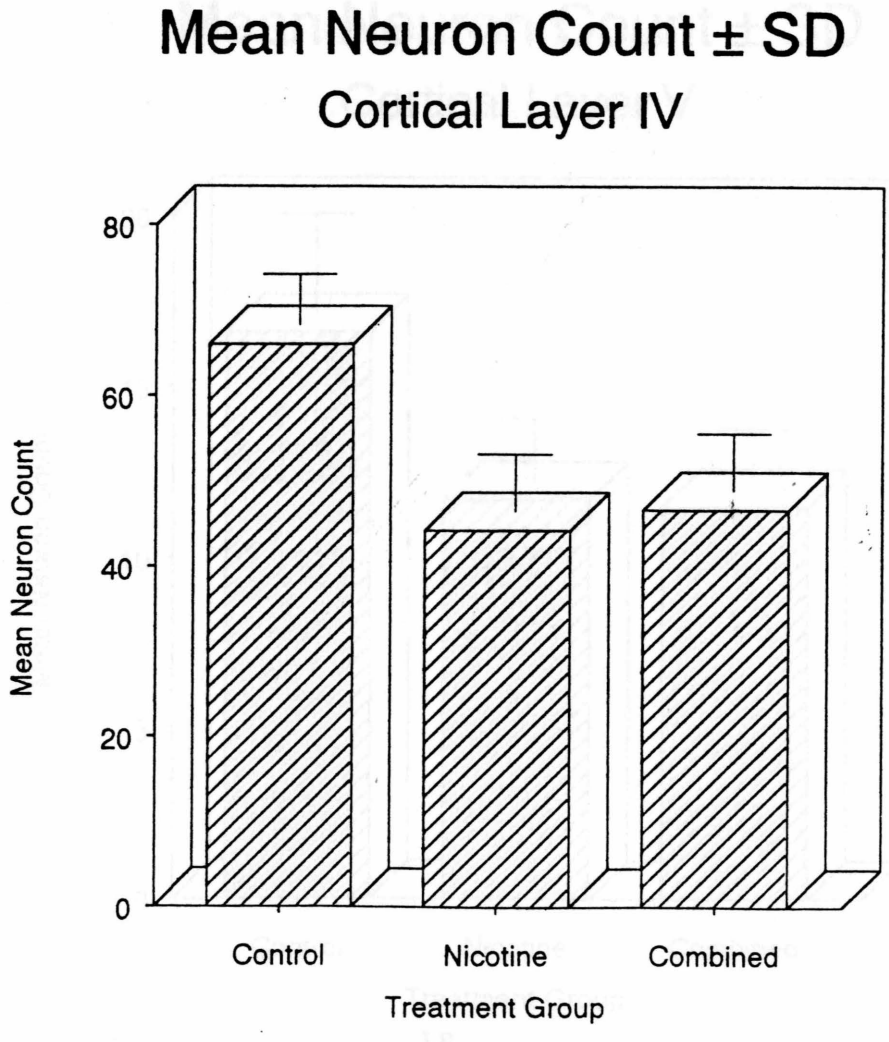
Treatment Group	Number in Group	Mean Brain-to-Body Weight Ratio ± SD
Control	3	0.055 ± 0.015
Nicotine	6	0.046 ± 0.008
Ethanol + Nicotine	5	0.041 ± 0.006

Table 3: Mean brain-to-body weight ratios for day 16 chick embryos in the control, nicotine treated, and ethanol + nicotine treated experimental groups. The number of chick embryos in each group is also noted. A t-test showed no significant differences between the control and nicotine treated groups ($t = -1.239$, $p > 0.01$) or the nicotine treated and the ethanol + nicotine treated groups ($t = 1.256$, $p > 0.01$). A moderately significant difference was found between the control and ethanol + nicotine treated groups ($t = 1.973$, $p < 0.05$).



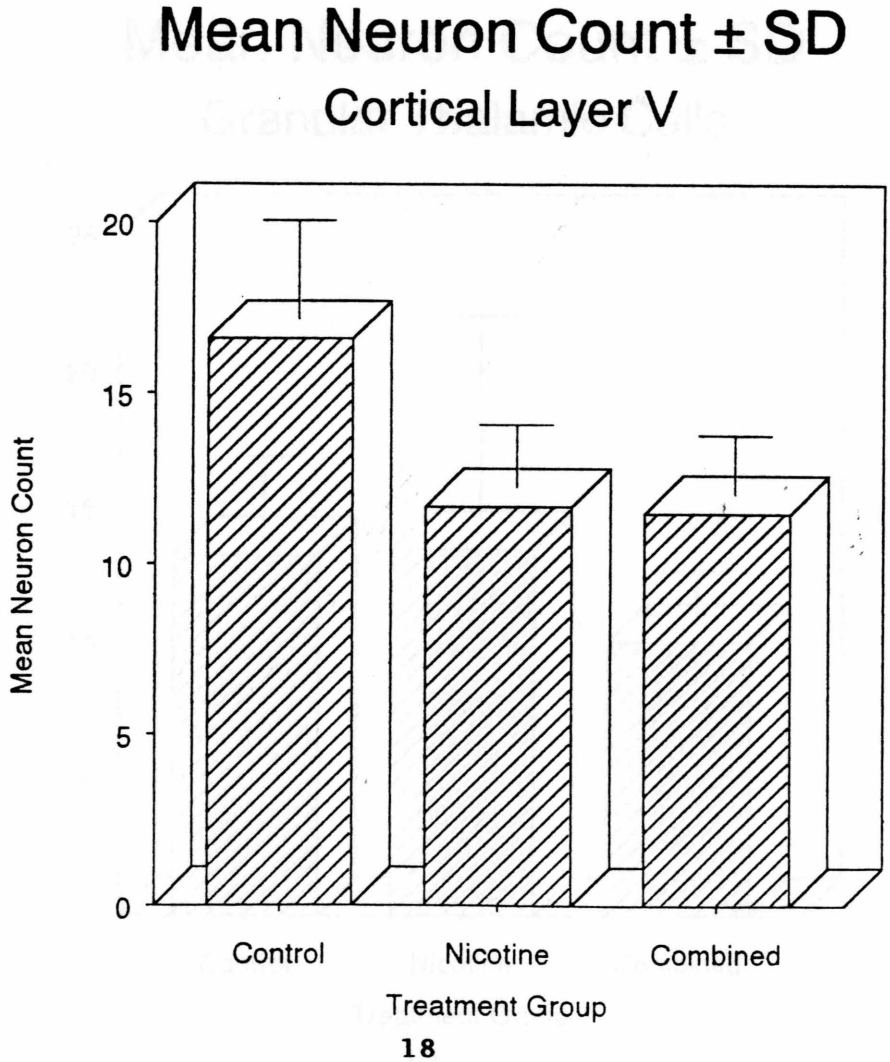
Treatment Group	Number of Cell Counts	Mean Neuron Count ± SD
Control	10	66.000 ± 5.831
Nicotine	10	44.200 ± 6.613
Ethanol + Nicotine	30	46.633 ± 6.682

Table 4: Mean neuron counts observed within 1964 μm^2 areas of cortical layer IV and the number of cell counts performed for the three treatment groups. A t-test showed significant differences between the control and nicotine treated groups ($t= 7.819$, $p< 0.01$) and the control and ethanol + nicotine treated groups ($t= 8.171$, $p< 0.01$). No significant difference was found between the nicotine and ethanol + nicotine treated groups ($t= -1.000$, $p> 0.01$).



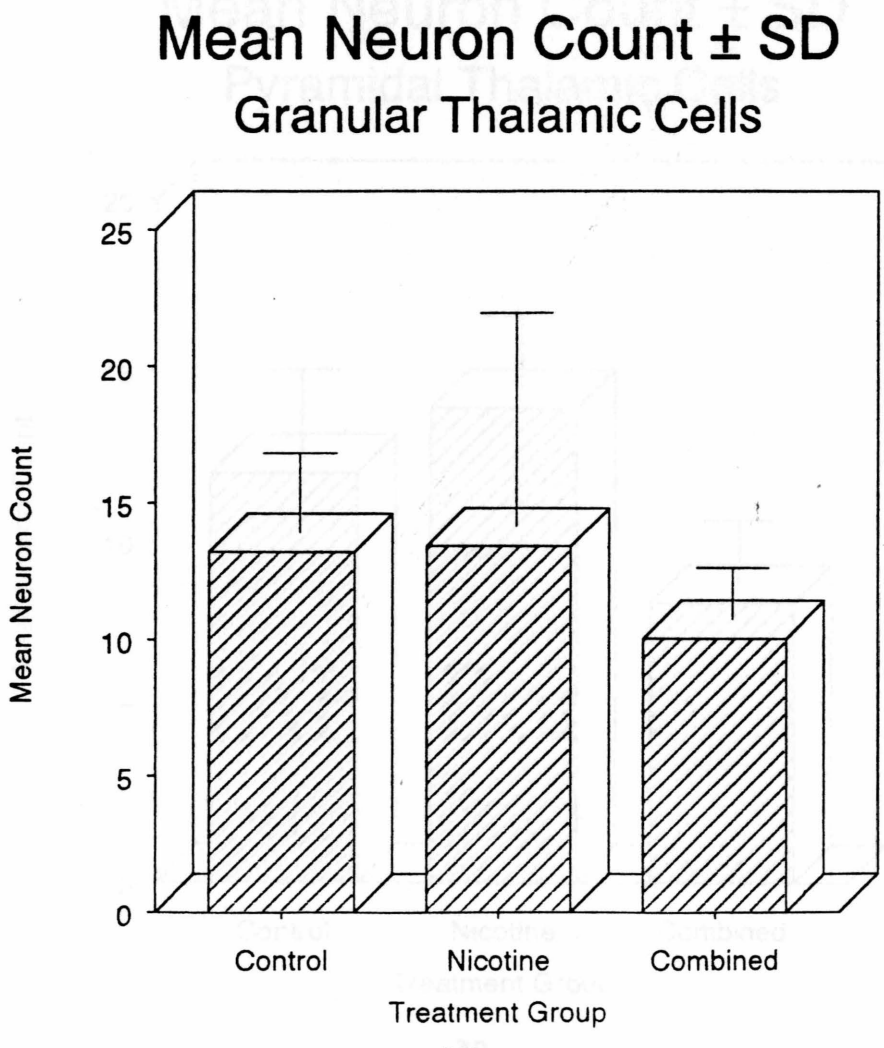
Treatment Group	Number of Cell Counts	Mean Neuron Count ± SD
Control	10	16.600 ± 2.875
Nicotine	10	11.700 ± 1.829
Ethanol + Nicotine	30	11.500 ± 1.717

Table 5: Mean neuron counts observed within 1964 μm^2 areas of cortical layer V and the number of cell counts performed for the three treatment groups. A t-test showed significant differences between the control and nicotine treated groups ($t= 4.547$, $p< 0.01$) and the control and ethanol + nicotine treated groups ($t= 6.809$, $p< 0.01$). There was no significant difference between the nicotine and ethanol + nicotine treated groups ($t= 0.314$, $p> 0.01$).



Treatment Group	Number of Cell Counts	Mean Neuron Count ± SD
Control	10	13.200 ± 2.898
Nicotine	30	13.400 ± 7.815
Ethanol + Nicotine	30	10.033 ± 1.866

Table 6: Mean neuron counts (granular cells) observed within 1964 μm^2 areas of the thalamic area and the number of cell counts performed for the three treatment groups. A t-test showed no significant difference between the control and nicotine treated groups ($t = -0.079$, $p > 0.01$). Significant differences were found between the control and ethanol + nicotine treated groups ($t = 4.023$, $p < 0.01$) and the nicotine and ethanol + nicotine treated groups ($t = 2.295$, $p < 0.01$).



Treatment Group	Number of Cell Counts	Mean Neuron Count ± SD
Control	10	12.000 ± 2.449
Nicotine	30	13.900 ± 2.107
Ethanol + Nicotine	30	8.200 ± 1.846

Table 7: Mean neuron counts (pyramidal cells) observed within 1964 μm^2 areas of the thalamic area and the number of cell counts performed for the three treatment groups. A t-test showed significant differences between the control and nicotine treated groups ($t= -2.373$, $p< 0.01$), the control and ethanol + nicotine treated groups ($t=5.190$, $p< 0.01$), and the nicotine and ethanol + nicotine treated groups ($t= 11.147$, $p< 0.01$).

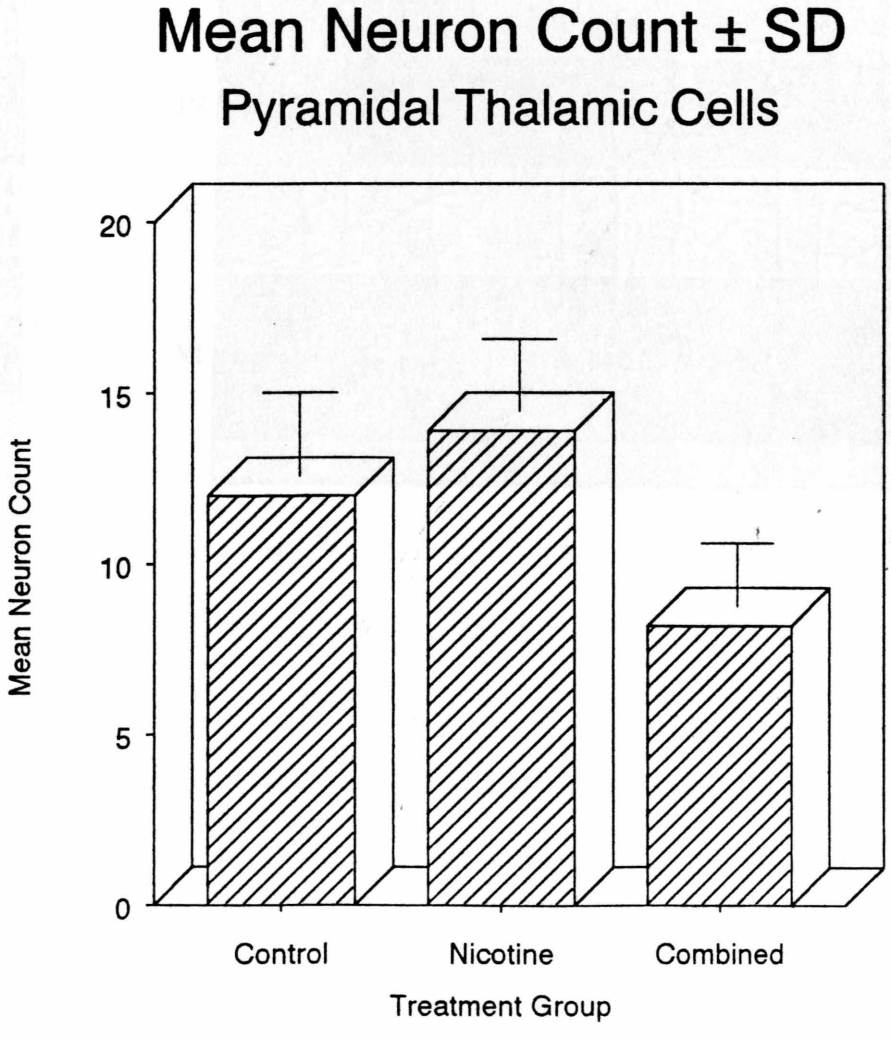
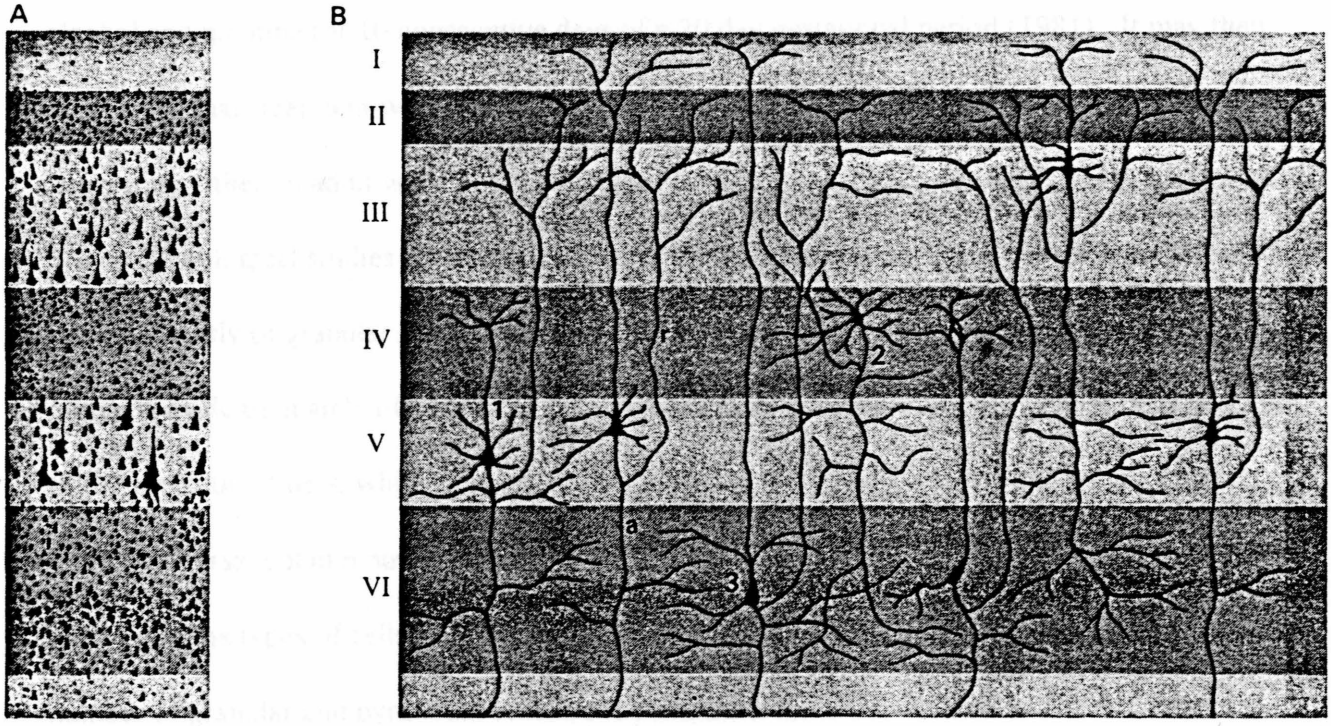


Figure 1: Illustration of the six cortical layers and the types of cells contained within each layer. Layer IV contains mainly granular cells, while Layer V consists mostly of pyramidal cells (Kandel and Schwartz, 1981).



V. Discussion

Although the mean brain-to-body weight ratios of the three treatment groups differed, only the difference between the control and the ethanol + nicotine treated groups was found to be moderately significant. This suggests that the combined effects of ethanol and nicotine have a more potent embryonic effect than the singular effect of nicotine. The effects of the combined daily treatments given during what corresponds to the first trimester of human gestation support the work of Peterson et al., who found the most significant fetal damage in rats exposed to both alcohol and nicotine for 10 consecutive days of a 20 day gestational period (1981). It may then be inferred that treatment with ethanol and nicotine results in the most serious fetal damage, suggesting either an additive or synergistic effect.

Histological studies were performed on cortical layers IV and V. Cortical layer IV consists mostly of granular cells and acts as a receiving area for the thalamus. Layer V of the cortex is made up mainly of Betz cells (pyramidal cells of the motor cortex). These cells give rise to the projection fibers, which connect to subcortical structures such as the brain stem, spinal cord, and dorsal column nuclei (Kandel and Schwartz, 1981). Figure 1 illustrates the layers of the cortex and the types of cells found within each layer. Comparisons were also made between the number of granular and pyramidal cells in the thalamic area.

Significant differences between mean neuron counts were found for the control and nicotine groups for both cortical layers. The number of pyramidal thalamic cells between the two groups also differed significantly. The ethanol + nicotine treated group differed significantly from the control group for all four neuron counts. The nicotine and ethanol + nicotine treated groups differed significantly for both of the thalamic neuron counts. Again, this suggests that the combined effect of ethanol and nicotine results in more brain damage than the effect of nicotine

only. Of the treated groups, the ethanol + nicotine treated group exhibited the most overall damage. The brains in this group had fewer cells per unit area, which could explain the moderately significant difference in brain-to-body weight ratios between this group and the control group. Decreased cell density in cortical layer V would result in fewer sub-cortical connections and impaired motor function. The fewer number of cells in the other areas would most likely result in overall suppressed brain function.

These findings suggest that nicotine induces brain damage by inhibiting the initial division of neuroblasts. The observation of fewer cells in the treated groups is one explanation for the lower brain-to-body weight ratios of the treated groups. The ethanol + nicotine treated group showed more marked effects regarding brain-to-body weight ratios and neuronal cell counts, which supports the hypothesis of an additive or synergistic effect. It has been documented that human infants exposed to both alcohol and nicotine in utero had lower weights at eight months of age than infants whose mothers abused only one of the substances (Barr et al., 1984). The results of this study illustrate the importance of abstaining from substance abuse during pregnancy. The grave effects that may result from the abuse of alcohol and nicotine during pregnancy must be stressed by caregivers. This damage could be avoided if mothers were educated about the subject and willing to change their lifestyles for the sake of their unborn babies. The treatments were given in what is analogous to the first trimester of human gestation. This study supports the claim that abuse of both alcohol and nicotine can have detrimental effects on the developing brain of the fetus. Previous studies (Gorman, 1992) have not found differences in neuronal cell counts between control and ethanol treated groups. This suggests that microencephaly due to the lack of neuroblast proliferation is a result of the nicotine treatment and compounded by the addition of alcohol.

VI. References

Abma, J.C., Mott, F.L. Substance use and prenatal care during pregnancy among young women. Family Planning Perspectives. 23: 117-128, 1991.

Armstrong, B.G., McDonald, A.D., Sloan, M. Cigarette, alcohol, and coffee consumption and spontaneous abortion. American Journal of Public Health. 82: 85-87, 1992.

Barr, H.M., Streissguth, A.P., Martin, D.C., Herman, C.S. Infant size at 8 months of age: relationship to maternal use of alcohol, nicotine, and coffee during pregnancy. Pediatrics. 74: 336-341, 1984.

Brooke, D.G., Anderson, H.R., Bland, J.M., Peacock, J.C., Stewart, C.M. Effect on birthweight of smoking, alcohol, caffeine, socioeconomic factors, and psychosocial stress. British Medical Journal. 298: 795-801, 1989. Cited in: Olsen, J., de Costa Pereira, A., Olsen, S.F. Does maternal tobacco smoking modify the effect of alcohol on fetal growth? American Journal of Public Health. 81: 69- 73, 1991.

Campbell, A.M. Effect of excessive cigarette smoking on maternal health. American Journal Obstetrics and Gynecology. 31: 502-508, 1936. Cited in: Condon, J.T., Hilton, C.A. A comparison of smoking and drinking behaviours in pregnant women: who abstains and why. The Medical Journal of Australia. 148: 381-385, 1988.

Campbell, A.M. Excessive cigarette smoking in women and its effect upon their reproductive efficiency. Journal of the Michigan Medical Society. 34: 146-151, 1935. Cited in: Condon and Hilton, 1988, as above.

"Carolina Fertile Chick Eggs Handling Instructions." Carolina Biological Supply Company (Burlington, NC: 1989).

Condon, J.T., Hilton, C.A. A comparison of smoking and drinking behaviours in pregnant women: who abstains and why. The Medical Journal of Australia. 148: 381-385, 1988.

Edwards, G. Alcohol and advice to the pregnant woman. British Medical Journal. 286: 247-248, 1983. Cited in: Condon and Hilton, 1988, as above.

Feng, T. Substance abuse in pregnancy. Current Opinion in Obstetrics and Gynecology. 5: 16-23, 1993.

Fried, P.A., O'Connell, C.M. A comparison of the effects of prenatal exposure to tobacco, alcohol, cannabis, and caffeine on birth size and subsequent growth. Neurotoxicology and Teratology. 9: 79-85, 1987.

Gorman, C.J. An investigation of the effects of "binge" v. "chronic" ethanol exposure on the brain weight of the embryonic chick: a model for brain damage associated with fetal alcohol syndrome. Honors Thesis: Washington and Lee University, 1992.

Gusella, J.L., Fried, P.A. Effects of maternal social drinking and smoking on offspring at 13 months. Neurobehavioral Toxicology and Teratology. 6: 13-17, 1984.

Jones, K.L., Smith, D.W., Ulleland, C.N., Streissguth, P. Pattern of malformation in offspring of chronic alcoholic mothers. Lancet. 1: 1267-1271, 1973. Cited in: Condon and Hilton, 1988, as above.

Kandel, E.R., Schwartz, J.H. Principles of Neural Science. Elsevier/North-Holland, Inc.: New York, 1981.

Landesman-Dwyer, S., Emanuel, I. Smoking during pregnancy. Teratology. 19: 119-125, 1979. Cited in: Persaud, T.V.N. Further studies on the interaction of ethanol and nicotine in the pregnant rat. Research Communications in Chemical Pathology and Pharmacology. 37: 313-316, 1982.

Lehtovirta, P., Forss, M., Rauramo, I., Kariniemi, V. Acute effects of nicotine on fetal heart rate variability. British Journal of Obstetrics and Gynaecology. 90:710-715, 1983. Cited in: Feng, 1993, as above.

Lindenschmidt, R.R., Persaud, T.V.N. Effect of ethanol and nicotine in the pregnant rat. Research Communications in Chemical Pathology and Pharmacology. 27: 195-198, 1980. Cited in: Persaud, 1982, as above.

Longo, L.D. The biological effects of carbon monoxide on the pregnant woman, fetus, and newborn infant. American Journal of Obstetrics and Gynecology. 129: 69-103, 1977. Cited in: Feng, 1993, as above.

Luck, M., Nau, H. Exposure of the fetus, neonate and nursed infant to nicotine and cotinine from maternal smoking. New England Journal of Medicine. 311: 672, 1984. Cited in: Paulson, R.B., Shanfeld, J., Dean, J., Mullet, D., Fernandez, M., Paulson, J.O. Alcohol and smokeless tobacco effects on the CD-1 mouse fetus. Journal of Craniofacial Genetics and Developmental Biology. 12: 107-117, 1992.

Marbury, M.C., Linn, S., Monson, R., Schoenbaum, S., Stubblefield, P.G., Ryan, K.J. The association of alcohol consumption with outcome of pregnancy. American Journal of Public Health. 73: 1165-1168, 1983. Cited in: Abma and Mott, 1991, as above.

Mills, J.L., Granbard, B.I., Harley, E.E., Rhoads, G.G., Berendes, H.W. Maternal alcohol consumption and birthweight. Journal of the American Medical Association. 252: 1875-1879, 1984. Cited in: Olsen et al., 1991, as above.

Nieburg, P., Marks, J.S., McLaren, N.M., Remington, P.L. The fetal tobacco syndrome. Journal of the American Medical Association. 253: 2998-2999, 1985. Cited in: Paulson et al., 1992, as above.

Olsen, J., da Costa Pereira, A., Olsen, S.F. Does maternal tobacco smoking modify the effect of alcohol on fetal growth? American Journal of Public Health. 81: 69-73, 1991.

Paulson, R.B., Shanfeld, J., Dean, J., Mullet, D., Fernandez, M., Paulson, J.O. Alcohol and smokeless tobacco effects on the CD-1 mouse fetus. Journal of Craniofacial Genetics and Developmental Biology. 12: 107-117, 1992.

Persaud, T.V.N. Further studies on the interaction of ethanol and nicotine in the pregnant rat. Research Communications in Chemical Pathology and Pharmacology. 37: 313-316, 1982.

Peterson, K.L., Heninger, R.W., Seegmiller, R.E. Fetotoxicity following chronic prenatal treatment of mice with tobacco smoke and ethanol. Bulletin of Environmental Contamination and Toxicology. 26: 813-819, 1981. Cited in: Persaud, 1982, as above.

Powell-Griner, E., Rogers, R. Strong compounding effects of multiple risks during pregnancy on birthweight and infant survival. Social Science Research. 72: 49-54, 1987. Cited in: Abma and Mott, 1991, as above.

Prager, K., Malin, H., Spiegler, D., Van Natta, P., Placek, P. Smoking and drinking behavior before and during pregnancy of married mothers of live-born infants and stillborn infants. Public Health Reports. 99: 117-127, 1984. Cited in: Feng, 1993, as above.

Resnik, R., Brink, G.W., Wilkes, M. Catecholamine mediated reduction in uterine blood flow after nicotine infusion in the pregnant ewe. Journal of Clinical Investigation. 63: 1133-1136, 1979. Cited in: Feng, 1993, as above.

Riesenfeld, A., Oliva, H. The effect of nicotine and alcohol on the fertility and life span of rats. Acta Anatomica. 128:45-50, 1987.

Rind, P. Smokers are two times as likely as nonsmokers to drink while pregnant. Family Planning Perspectives. 23: 292, 1991.

Rosell, H.L. "Clinical Pharmacology of the Fetal Alcohol Syndrome." Biochemistry and Pharmacology of Ethanol. E. Majchrowicz, ed. Plenum Press: New York, 1974.

Simpson, W.J.A. A preliminary report on cigarette smoking and the incidence of prematurity. American Journal of Obstetrics and Gynecology. 73: 808-815, 1957. Cited in: Condon and Hilton, 1988, as above.

Sokol, R.J. Alcohol and abnormal outcomes of pregnancy. Canadian Medical Association Journal. 125: 143-148, 1981. Cited in: Condon and Hilton, 1988, as above.

Sontag, L.W., Wallace, R.F. The effect of cigarette smoking during pregnancy upon the fetal heart rate. American Journal of Obstetrics and Gynecology. 29: 77-83, 1935. Cited in: Condon and Hilton, 1988, as above.

Sulaiman, N.D., Floredy, Cdu.V., Taylor, D.J., Ogston, S.A. Alcohol consumption in dundee primigravidas and its effects on outcome of pregnancy. British Medical Journal. 296: 1500-1503, 1988. Cited in: Olsen et al., 1991, as above.

Tennes, K., Blackard, C. Maternal alcohol consumption, birthweight, and minor physical anomalies. American Journal of Obstetrics and Gynecology. 138: 774-780, 1980. Cited in: Abma and Mott, 1991, as above.

West, J.R. Fetal alcohol-induced brain damage and the problem of determining temporal vulnerability : a review. Alcohol Drug Research. 7: 423-441, 1987.

Zajac, C.S., Abel, E.L. Animal models of prenatal alcohol exposure. International Journal of Epidemiology. 211: S24-S32, 1992.

Zorzano, A., Herrera, E. Disposition of ethanol and acetaldehyde in late pregnant rats and their fetuses. Pediatric Research. 25: 102-106, 1989. Cited in : Paulson et al., 1992, as above.

VII. Additional References

Elipoulos, C., Klein, J., Phan, M.K., Knie, B., Greenwald, M., Chitayat, P., Koren, G. Hair concentrations of nicotine and cotinine in women and their newborn infants. Journal of the American Medical Association. 271: 621-623, 1994.

Hahn, G.M., Shiu, E.C., Auger, E.A. Mammalian stress proteins HSP70 and HSP28 coinduced by nicotine and either ethanol or heat. Molecular and Cellular Biology. 11: 6034- 6040, 1991.

Kandall, S.R., Gaines, J. Maternal substance use and subsequent sudden infant death syndrome (SIDS) in offspring. Neurotoxicology and Teratology. 13: 235-240, 1991.

Little, R.E., Uhl, C.N., Labbe, R.F., Abkowitz, J.L., Phillips, E.L.R. Agreement between laboratory tests and self-reports of alcohol, tobacco, caffeine, marijuana, and other drug use in post-partum women. Social Science & Medicine. 22: 91- 98, 1986.

Martin, J.C., Martin, D.C., Chao, S., Shores, P. Interactive effects of chronic maternal ethanol and nicotine exposure upon offspring development and function. Neurobehavioral Toxicology and Teratology. 4: 293-298, 1982.

Riesenfeld, A. Growth-depressing effects of alcohol and nicotine in two strains of rats. Acta Anatomica. 122: 18-24, 1985.

Sloan, L.B., Gay, J.W., Snyder, S.W., Bales, W.R. Substance abuse during pregnancy in a rural population. Obstetrics & Gynecology. 79: 245-248, 1992.

Staisey, N.L., Fried, P.A. Relationship between moderate maternal alcohol consumption during pregnancy and infant neurological development. Journal of Studies on Alcohol. 44: 262-270, 1983.

Walpole, I., Zubrick, S., Pontre, J. Is there a fetal effect with low to moderate alcohol use before or during pregnancy? Journal of Epidemiology and Community Health. 44: 297-301, 1990.