# THE EFFECT OF SOUND
# STIMULATION DURING
# GESTATION ON THE BEHAVIOR
# OF RAT OFFSPRING

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# THE EFFECT OF SOUND STIMULATION DURING GESTATION ON THE BEHAVIOR OF RAT OFFSPRING

by

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A Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Department of Psychology
Memorial University of Newfoundland
August 1978

St. John's Newfoundland

# ABSTRACT

The role of a fetal response (direct mediation) in the effects of sound stimulation during gestation on the postnatal behavior of rat offspring was studied. By deafening the mother and so eliminating a maternal stress response to sound stimulation, conditions were optimized for examining direct effects.

Prior to mating, female hooded rats were either experimentally deafened or left intact (hearing condition). Beginning on the 13th day of gestation, some of the deaf mothers and some of the hearing mothers were presented with an 85 db (re $20\mu N/m^2$) white noise stimulus. Another hearing group received a low noise stimulus, a 65 db (re $20\mu N/m^2$) white noise. The remaining deaf and hearing mothers received the same experimental treatment but without the noise presentation. Experimental treatments continued through the 19th day of gestation. An undisturbed control group was also included in the design. A direct effect of noise stimulation would have to be considered if the deaf noise group differed significantly from its control group, deaf no noise.

Offspring were tested on three behavioral tests: open-field, shuttle box avoidance, and one-way avoidance. In the open-field, the hearing noise group ambulated significantly more than the control group. All groups ambulated more than the controls. The shuttle box yielded insignificant results. On the one-way avoidance task, response latency scores on a CS pre-exposure were similar to group ambulation scores.

An extinction effect occurred only in the hearing noise group which had not extinguished in 200 trials.

The results of these tests were interpreted as indicating that noise stimulation is predominantly indirectly mediated. An important factor in the treatment effect was the placement of the maternal cages into the experimental chamber. This raised the question of the role of differing levels of novelty in the prenatal stress effect, particularly as this difference may account for contradictory findings in the prenatal stress literature.

# ACKNOWLEDGEMENTS

I would like to express sincerest gratitude for the patience and helpful advice of Dr. John Evans, my supervisor. Without his support, this work would never have been finished. I also thank him for the opportunity to work as a research assistant. Thanks are extended to Drs. Carolyn Harley and Norman Braveman for their constructive, critical comments.

Without the help of Les Samuelson, the testing of the rats in the open-field would have been impossible. I also thank Joan Crane for her company during those long hours at the Vivarium. She provided relief from the rats. I want to express appreciation for the help of Dick Noseworthy who made the maintenance of 153 rats possible.

Steven Hughes helped in the final preparation of this thesis. He was a patient proof reader and a tremendous help in the preparation of the figures.

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# INTRODUCTION

Research on prenatal stress is generally concerned with the effects that events occurring during gestation have on later postnatal development of the offspring. Of particular interest is the effect that maternal stress has on the behavioral development of the offspring. However, before the importance of a maternal stress response can be established, the ability of the fetus to respond to the stimuli used to induce maternal stress needs to be established (Ferreira, 1965).

The maternal and fetal component of prenatal stress can be conceived as representing differences in mediation: indirect and direct effects (Joffe, 1969). An indirect effect is one which is mediated by the mother and transmitted to the fetus via the placental blood barrier. A direct effect is one in which the fetus responds to, or is affected by, the physical characteristics of the stimulus which need not affect the mother. An example is X-irradiation which can have massive effects on the fetus at levels which are not harmful to the mother (Furchtgott, 1963; Joffe, 1969).

Even though there are two ways in which prenatal effects can be mediated, the assumption in the prenatal stress literature is that the experimental manipulations are indirectly mediated (Archer & Blackman, 1971). Studies of the effects of using neutral stimuli as stressors (Morra, 1965b; Jolley & Dreesman, 1973), however, have raised the question as to whether the observed behavioral effects are the result of direct effects. Specifically, the question arises as to whether sound stimulation has direct effects on the fetus.

The experiments to be presented in this thesis examine the relative importance of direct effects of sounds on the behavioral development of rat offspring. Even though it is not possible to completely bypass the maternal environment, it is still possible to assess the relative importance of direct effects on the behavioral development of the offspring. First, a review of the literature of indirect and direct effects is presented.

## Literature Review

Evidence for indirect effects comes from two bodies of data: experimental manipulations which are considered stressful to the mother and experimental manipulations which alter the maternal hormonal stress response. Stressful manipulations of the mother are confined to stimuli which while not resulting in tissue damage to the mother do result in a maternal psychological stress response (Archer & Blackman, 1971). Since this method is more frequently employed, this literature will be considered first.

The bulk of the literature dealing with experimental manipulations considered stressful to the mother use the conditioned avoidance paradigm. This technique was first used by Thompson (1957a). Basically, the design was as follows. Two groups of rats were used: One group was an undisturbed control group, and one group was the stressed group. Prior to mating, the stressed group learned a shuttle box avoidance task. During gestation, this group was presented with only the CS, a buzzer, and with the avoidance response blocked. This procedure continued daily until the birth of the litters. At birth, all litters, experimental and control, were fostered in such a way that one-third were raised by their biological mother, one-third were raised by different mothers in the same condition, and one-third were fostered out to mothers in the opposite condition. At 30-40 days of age, the offspring were tested in an open-field for three daily 10 minute sessions and later in an emergence from home cage test. Behaviors recorded in the open-field were latency of activity and number of squares crossed. Compared to the control group, the offspring of the experimental mothers ambulated less and had longer latencies. Latency to leave the home cage and latency to reach food at the end of a runway were scored in the emergence from home cage test. Compared to the controls, the experimentals had higher latencies on both emergence and reaching the food. At 130-140 days of age, these tests were repeated. The direction of the differences in the open-field did not change with age. The differences for latency for emergence were not significant. There were no fostering effects at either age. The patterning of results, high latency and low ambulation in the experimental offspring, was interpreted as indicative of greater emotionality.

This stress procedure, conditioned avoidance, has been used in other prenatal studies (Ader & Belfer, 1962; Hockman, 1961; Joffe, 1969; Morra, 1965a, 1965b; Thompson, Watson, & Charlesworth, 1962). There is general agreement among these studies. The offspring of stressed mothers are more emotional than a control group. Despite the consistency of the data, there are two procedural problems inherent in the conditioned avoidance procedure: premating stress and the use of a buzzer as the CS.

The study of prenatal stress effects is supposedly confined to events occurring <u>during</u> gestation. The conditioned avoidance procedure, however, involves the acquisition of an avoidance response prior to mating. This premating experience is itself stressful and could contribute to the prenatal stress effect (Kaplan, 1957). The premating stress may either alter the maternal hormonal environment prior to as well as during gestation or it may lower the maternal "threshold of reactivity" to environmental stimuli during gestation (Thompson, 1957b).

Both the studies of Ader and Belfer (1962), and Joffe (1965) were addressed to the problem of premating stress. Ader and Belfer (1962) found significant differences in open-field ambulation between a premating stress only and a premating plus gestational stress group. The latter group had lower ambulation scores and fewer center circle entries. Their study, however, lacked an undisturbed control group. Without this group, it was not possible to determine if premating stress had an effect relative to a control group. On the other hand, Joffe (1965) showed that depending upon the behavioral measure used, premating stress did have an effect. On open-field ambulation, the premating stress group differed significantly from both the premating stress plus gestational stress group and the control group. Depending upon the paternal genotype, premating stress either increased or decreased open-field ambulation. On an avoidance task, only the premating plus gestational stress group scored significantly more avoidances. Though Joffe's study is the only one to examine premating stress with the use of proper controls, there is supportive physiological evidence for a premating stress effect. Active avoidance elevates corticosterone basal levels for periods beyond training (Brush & Froehlich, 1975; Wertheim, Conner, & Levine, 1969). Further studies would be necessary to demonstrate that such a change could affect the fetus. The point to emphasize from the work of Joffe (1965) is that postnatal effects may be the result of conditions instituted prior to as well as during gestation. Until further appropriately controlled studies are done, caution must be exercised in concluding that the prenatal stress effect is due solely to events occurring during gestation.

Just as premating stress may alter the maternal environment during gestation, so the experiences during gestation may alter the maternal component of the mother/ offspring social interactions that occur prior to weaning. This altered postnatal environment may be contributing to the effects of prenatal stress. Controlling for postnatal effects necessitates the use of a fostering procedure. There is conflicting experimental evidence, however, as to whether prenatal treatment alters the later postnatal social environment. One series of experiments indicate that there are no behavioral differences between offspring in the same treatment which were raised by mothers with different gestational experiences: stressed or undisturbed (Ader & Belfer, 1962; Thompson et al., 1962). Thompson et al. (1962) found there was a tendency for a greater difference in ambulation scores between prenatally stressed and control offspring if both groups were raised by control mothers. However, the direction of the difference remained the same regardless of the fostering condition. Another series of experiments indicate that there is a postnatal maternal effect of prenatal stress (Ader & Conklin, 1963; Hockman, 1961; Joffe, 1969). Contrary to the previously cited experiments, behavioral measures of offspring from the same treatment were affected by the experimental history of the foster mother.

Despite the conflicting viewpoints concerning fostering, the data actually present a cogent argument for fostering. The enhanced differences between treatment groups that were fostered out to controls indicate that there is an interaction between the biological mother and her offspring which counteracts the experimental manipulations. In the extreme case, the lack of fostering results in no between treatment differences (Hockman, 1961). Even if the offspring contribute to the altered social interactions, by ensuring a uniform maternal environmental background, any differences between groups can only be attributed to differences in prenatal treatment.

Whereas fostering is a technical problem for any prenatal stress design, there is another procedural problem which is confined to the conditioned avoidance design: the predominant use of buzzer as the CS. The possibility of direct effects of the buzzer on the developing fetus has not received much experimental attention. This will be considered later. That the effects of conditioned avoidance are not dependent upon the use of a buzzer as the CS was illustrated by Joffe (1969). A light was used as the CS. The change in the CS did not alter the results: the prenatally stressed group was more emotional.

Another frequently used method for creating prenatal stress is handling of the mother during gestation. Females are handled daily from the onset of gestation until the birth of their litters. The daily length of handling can be as little as 3 minutes a day (Ader & Plaut, 1969) or as long as three 10 minute sessions per day (Ader & Conklin, 1963). Regardless of the daily length of handling, the trend is for the offspring of handled mothers to be less emotional than an undisturbed control group. This effect is in the opposite direction from the previously cited conditioned avoidance literature in which stress resulted in greater emotionality. Though both procedures are stressful, there must be a quantitative or qualitative difference in the maternal stress response which differentially affects the developing fetus.

The other supportive evidence for indirect effects comes from studies which have examined the effects of the administration of hormones associated with the stress response during gestation. One approach has been the injection of epinephrine or norephinephrine (Thompson, Goldenberg, Watson, & Watson, 1963; Thompson et al., 1962; Young, 1963, 1964). In general either one of these hormones produces results which mimic the conditioned avoidance procedure: the offspring are more emotional. However, in three of these studies (Thompson et al., 1962; Young, 1963, 1964), the prenatal treatment is confounded with postnatal conditions due to the lack of a fostering procedure. The injection procedure itself has certain drawbacks. Depending on whether a control substance, distilled water or isotonic saline, is injected intraperitoneally or subcutaneously, the control substance may have effects on the offspring (Havlena & Werboff, 1963). This may apply to any substance. There is one other problem which needs to be examined: the effect of saline. In Thompson et al. (1963), saline had an effect similar to epinephrine though this depended on the trimester of administration. A saline effect was more pronounced when body and adrenal weights were examined (Thompson & Goldenberg, 1962). What needs to be emphasized from these studies is that the occurrence of such an effect means that the type of substance injected may not be important. The occurrence of an injection during gestation may be the important factor. Even though the substance injected does have some effect since there are differences between saline, epinephrine, and norepinephrine (Thompson et al., 1963; Young, 1963, 1964), it is difficult to assert that it is the substance rather than an interaction of the substance and the injection procedure which is producing effects.

There are ways of altering the maternal hormonal levels without using an injection procedure. The next two studies did not use injections but resulted in alterations in maternal hormonal levels. These studies also have implications for one of the proposed mediating systems of prenatal stress: the pituitary-adrenal axis. The pituitary-adrenal axis is involved in early postnatal manipulations (Levine & Mullins, 1968) and is activated under stressful conditions, hence it could be responsible for prenatal stress effects (Archer & Blackman, 1971).

In one study (Joffe, Milkovic, & Levine, 1972) maternal corticosterone levels were altered. One group was adrenalectomized; the other group had a tumor implanted which secreted ACTH. In the former case, ACTH levels were high but no glucocorticoids were available. In the group with the tumor, both ACTH and glucocorticoid levels were high. Despite the functional difference between these groups, both groups ambulated more than controls when tested in the open-field. On an avoidance task, only the adrenalectomized group had a significantly greater number of avoidances. In another study (Smith, Joffe, & Heseltine, 1975), one group had high ACTH/low glucocorticoid levels, another group had low ACTH/low glucocorticoid levels, and another group had a completely functional pituitary-adrenal system. In addition, half of each group were given conditioned avoidance training prior to mating and CS presentations during gestation. The manipulations of the pituitary-adrenal axis affected the ambulation scores in the open-field. The stress procedure, however, affected both ambulation scores and performance on an avoidance task. The magnitude and direction of the effect depended upon the treatment group. In both studies, manipulations of the pituitary-adrenal axis affected the offsprings' behavior. The findings, however, are conflicting. In the first study, differences in ACTH/glucocortoid levels did not differentially affect open-field behavior. In the second study, differences in ACTH/glucocortoid levels did differentially affect open-field behavior. Furthermore, stress effects were found in conditions which according to Smith et al. (1975), the maternal pituitary-adrenal system "should have been incapable of responding to stress." Even though adrenalectomy does not prevent further ACTH response to stress (Brush & Froehlich, 1975; DiGuisto, Cairncross, & King, 1971), these two studies do raise doubts as to the importance of the pituitary-adrenal system in prenatal stress effects.

Despite the shortcomings of some of the studies reviewed, the evidence for indirect effects can be summarized as follows: Conditioned avoidance training and maternal handling during gestation alter later postnatal emotional behavior. These two procedures, while they do not inflict physical damage on either the mother or the fetus, are psychological stressors. They both elicit a corticosterone response of the pituitary-adrenal axis (Johnston, Miya, & Paolino, 1974) which is considered to be the main system mediating prenatal effects (Archer & Blackman, 1971). Furthermore, there is evidence that radioactively labeled corticosterone, 14-C-4, does cross the placental barrier (Zarrow, Philpott, & Denenberg, 1970). There is also supportive physiological evidence that alterations in corticosterone levels mimic behavioral manipulations of the mother, though the pituitary-adrenal axis may not be the sole system involved in prenatal stress. Other hormones associated with the stress response, epinephrine and norepinephrine, are also effective. In conclusion, there is experimental evidence available which supports an indirect mediation process whereby maternal experiences can influence the behavioral development of offspring.

In contrast to the supportive data for indirect effects, the evidence for direct effects is less well-documented. The importance of direct effects lies in the emphasis usually given to the maternal reaction mediating the stress effect. If attention is to be focused on possible maternal hormones influencing fetal development, it must first be established that some physical property of the stimulus is not directly influencing the fetus. An example of a stimulus having direct effects is X-irradiation. Though there is some dispute in the literature as to whether there is a maternal component in X-irradiation effects (Meier, 1961), it is fairly well-documented that X-irradiation at levels which do not adversely affect the mother results in physical as well as behavioral abnormalities in the offspring

(Furchtgott, 1962, 1963). Even though X-irradiation does result in physiological changes (Furchtgott, 1963; Levy, Carroll, Smith, & Hofer, 1974), it is not a "psychological stressor" in the sense that it requires external perception of the stimulus. Since prenatal stress is concerned with the effects of psychological stressors, X-irradiation will not be considered further.

Another stimulus which may have direct effects on the developing fetus is sound. This possibility is important for prenatal stress literature, because a buzzer is commonly used as a CS in the conditioned avoidance procedure. The effect of the buzzer presentations is presumed to be indirect due to the previously learned avoidance response. However from studies of human pregnancies, it is known that sound can be transmitted through the maternal body wall (Bench, 1968). There is also anecdotal as well as experimental evidence indicating that there is increased human fetal activity during sound presentations (Sontag, 1966). A problem arises as to whether this evidence is indicative of a direct effect of sound. A critical variable in the determination of a direct effect appears to be the difference between the time of sound stimulation and the fetal response. If the time lag between stimulus onset and fetal response is short, the fetal response is most likely the result of fetal perception of and response to sound stimulation. As the time lag increases, the fetal response may be due to a change in the maternal hormonal environment. The latter condition is an indirect effect. Sontag, Steele, and Lewis (1969) studied the time lag in sound presentation and changes in fetal and maternal heart rate. A change in maternal heart rate occurred 30 seconds after stimulus onset. A fetal response did not appear until 90 seconds after stimulus onset. The slowness of the fetal response was interpreted as reflecting an indirect effect. Though heart rate changes are most likely due to a maternal response, gross motor movement of the fetus may be a direct (fetal) response (Ferreira, 1965; Sontag et al., 1969).

These findings, however, do not offer much insight into whether sounds have direct or indirect effects on the rat fetus. The development of the rat auditory system differs from that of humans. The inner ear begins developing on the 12th day post-conception. The spiral ganglion develops between the 17th and 18th day. During the same period (17th and 18th day), the external meatus is completely filled with periderm (Altman & Dittmer, 1962). Postnatal recordings of cochlear potentials indicate no response to sounds until the 8-9th day (Crowley & Hepp-Reymond, 1966). Despite these structural limitations on the onset of an auditory response, the fetal response need not be auditory in nature: it could be tactual. This is an important consideration. In the case of human studies in which there is increased fetal activity during sound presentations, there is a controversy as to whether the response is auditory or tactile in nature (Carmichael, 1970). Until recordings are available of rat fetal activity the auditory limitations of the fetus do not negate the possibility of direct effects. Other systems may mediate a direct effect.

The problem of direct effects has been raised in the work of Morra (1965b), and Jolley and Dreesman (1973). Both of these studies have examined the effects of neutral stimuli as prenatal stressors. Previous stress paradigms used a conditioned avoidance procedure to induce maternal stress. The assumption was that to induce an anxiety response in the mother, it was first necessary to establish an association between a neutral stimulus and a noxious event. Morra, however, pointed out that any stimulus is capable of inducing fear "when it causes the organism to depart markedly from optimal levels of arousal" (Morra, 1965b). Thus the CS stimulus alone should produce behavioral effects in the offspring similar to the effects produced by the conditioned avoidance procedure.

Morra's experiment included three treatment conditions: a group which received avoidance training prior to mating and CS (a buzzer) presentations during gestation; a group which experienced no avoidance training but did receive buzzer presentations during gestation; and an equivalently handled control group. At 25 days of age, all the offspring were tested in an open-field. The offspring of the buzzer only group and the conditioned avoidance group had similar ambulation scores. Both groups were less active than the control group. This was interpreted as indicating greater emotionality in the two experimental groups. It was concluded that sound presentations in a novel environment are just as stressful as sound presentations previously associated with shock.

Morra's experiment is important in terms of the technique used to induce stress. The stress procedure can be confined to the gestation period which eliminates the problem of premating stress. Furthermore, stress intensity and period of administration are more easily controlled. This study, however, faces two problems. First, prenatal and postnatal conditions were confounded due to the lack of a fostering procedure. Second, there was no control for direct effects of sound on the fetus.

Jolley and Dreesman (1973) were interested in Morra's technique for inducing stress, however, there was concern with the possibility of direct effects of sound. To minimize possible direct effects on the fetus, Jolley and Dreesman (1973) used light as the neutral stimulus. Their evidence indicated that light has a low probability of penetrating through the hair and body wall of a rat. A prenatal effect would thus be a maternal effect. Experimental mothers were presented with 15.3 lux for 5 minutes for 14 consecutive days during gestation. A control group received .07 lux. The offspring were tested in an open-field. Recordings were made of ambulation, amount of defecation, and heart rate. Differences were found in defecation and heart rate. These results were replicated by Jolley and Adam (1975). In both instances, the offspring of the experimental mothers were less emotional than the controls. The magnitude of the effect while not large was reliable.

Though these studies (Jolley & Adam, 1975; Jolley & Dreesman, 1973; Morra, 1965b) used neutral stimuli, the effects on the open-field were not the same. Morra (1965b) found effects on latency and number of traversals. These were the only behaviors which were recorded. Jolley and Dreesman (1973), and Jolley and Adam (1975) measured heart rate, defecation, and number of traversals. Of these three measures, ambulation was the only variable which did not differentiate between the treatments. Whether the differences in open-field behavior can be attributed to the use of different stressors is not clear. Within the prenatal stress literature, the same prenatal stressor does not result in uniform effects on open-field behavior. Measuring both defecation and number of traversals in the open-field, studies which used the conditioned avoidance procedure have found either effects on both ambulation and defecation (Hockman, 1961; Thompson et al., 1962) or effects only on ambulation (Ader & Belfer, 1962; Joffe, 1969). Regardless of whether stress affects both defecation and ambulation or only one of these behaviors, the direction of the stress effect is consistent across studies. Stress increases defecation and decreases ambulation scores. These changes in open-field behaviors are interpreted as indicating an increase in emotionaltiy (Archer & Blackman, 1971). Rather than focusing on which behaviors in the open-field are affected by prenatal stress, attention is given as to whether prenatal stress increases or decreases emotional reactivity.

The importance of the differences in open-field behavior in the studies of Jolley and Adam (1975), Jolley and Dreesman (1973), and Morra (1965b) is the difference in emotionality. In one instance (Morra, 1965b), the offspring of experimental mothers were more emotional than controls. In contrast, the work of Jolley showed that the offspring of the experimental mothers were less emotional than controls so far as open-field defecation was concerned. This difference in the emotionality of the offspring may reflect either differences in the experimental procedure or differences between the neutral stimuli: sound versus light. There are two important differences in the experimental procedure. The first difference is the use of fostering. Morra (1965b) did not use a fostering procedure whereas Jolley did. The lack of a fostering control in Morra results in confounding prenatal and postnatal conditions. The second experimental difference is the amount of novelty during stimulus presentations. For the buzzer presentations, Morra placed the rats into the avoidance chamber. In contrast Jolley left the rats in their home cages during stimulus presentations. Introduction into a novel chamber itself results in an elevation in corticosterone levels (Bassett, Cairncross, & King, 1973). The reaction to a stimulus in a novel environment may differ from the reaction to a stimulus in a familiar environment. The remaining difference between the studies of Morra and Jolley is the difference in the neutral stimulus. Morra used a buzzer; Jolley used a light. The difference in the emotionality of the offspring may reflect qualitative/quantitative differences in the maternal hormonal response to sound and to light. On the other hand, the difference may be one of mediation. Since sound presentations may have direct effects on the fetus, the observed effect of sound presentations may reflect an interaction of direct and indirect mediation. Light presentations, however, produce effects only through indirect mediation.

Of the previously discussed differences between the studies of Morra and Jolley, two of them, fostering and novelty, can be experimentally controlled. Determining whether different neutral stimuli have different prenatal stress effects is a more difficult problem. Before any meaningful study can be made of qualitative/quantitative differences in the maternal hormonal response to light and sound, it first needs to be established whether sound does have direct effects on the fetus. It was the purpose of this thesis to examine the direct effects relative to indirect effects of sound on the behavior of rat offspring. A pilot study will be presented first.

## Pilot Study

The maternal/fetal system of mammals is a very closely integrated system. In order to examine the relative importance of direct effects, certain experimental procedures were used. First, rat mothers were experimentally deafened to minimize the maternal stress response to sound. One of the effects of sound presentations is an elevation in corticosterone levels. Deafening abolishes this stress response, indicating that the response is neurally mediated (Henkin & Knigge, 1963). Secondly, handling of the experimental mothers was held to a minimum. Handling, itself, is a prenatal stressor (Ader & Conklin, 1963; Ader & Plaut, 1968). After mating, the only disturbance experimental mothers were subjected to was transporting of the home cage into the experimental room. Using these two procedures, the maternal stress response to the stimulus as well as to the experimental procedure should be slight. Under these conditions, an effect of sound on the postnatal behavior of the offspring would be evidence for direct effects of sound on the fetus.

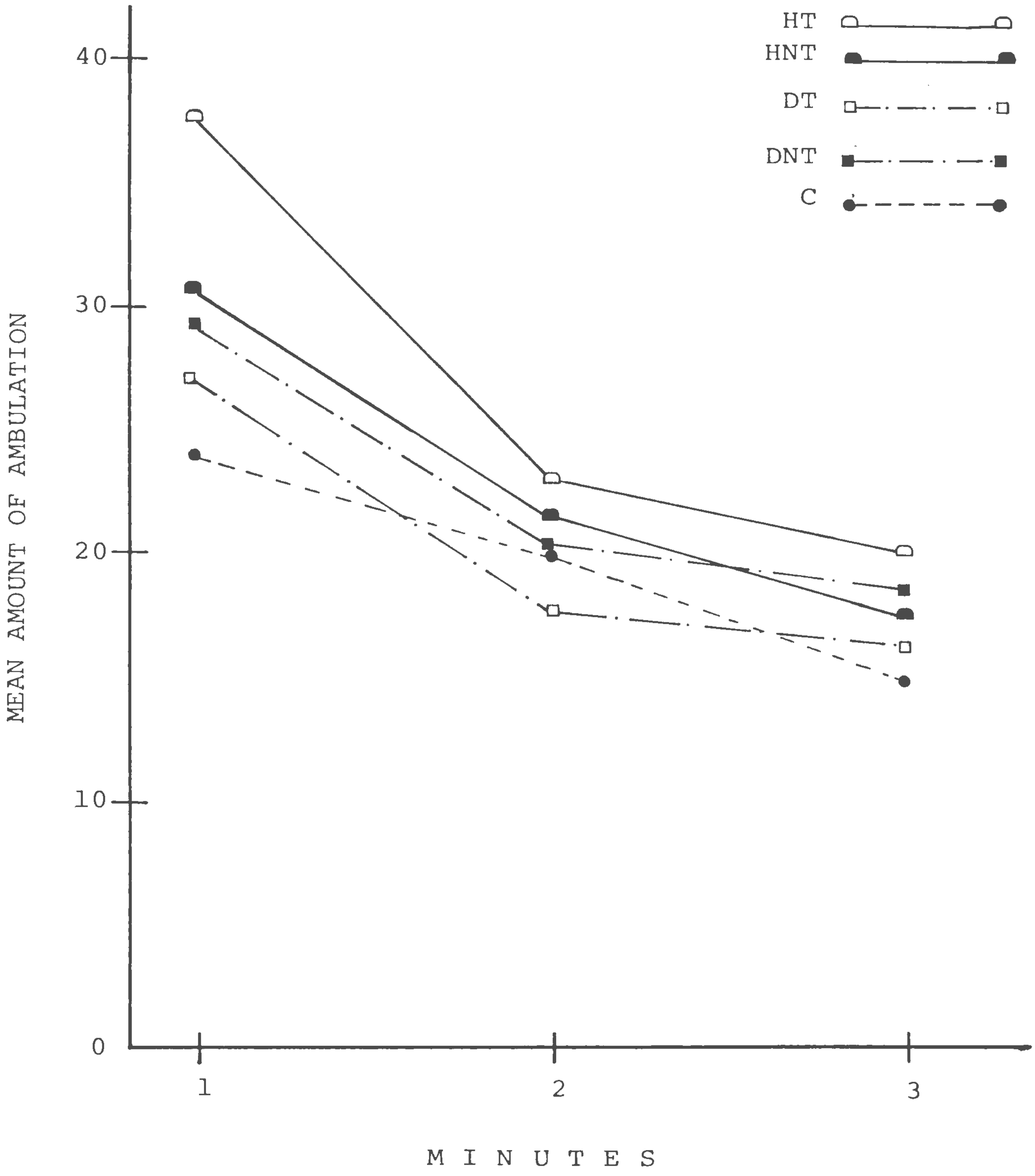
There were 26 experimental females, 28 foster mothers, and 8 undisturbed control mothers. The foster and control mothers were not disturbed during gestation. Of the experimental mothers, half were deafened prior to mating. Deafening was accomplished by putting an 18 gauge needle into the ear canal with the intention of breaking the tympanum and the ossicular chain of the middle ear. Collodium was then injected into the ear canal.

On the 13th day of gestation, the experimental mothers were carried in their home cages into the experimental room. Those rats in the tone presentation groups, hearing-tone and deaf-tone, received the following treatment. They were exposed to a 2.5 KHz tone of 3 second duration at an intensity of 85 db (re 20$\mu$N/m$^2$). There was a total of 3 tone presentations each spaced at 1 minute intervals. Rats were then returned to the holding room. The procedure was repeated an hour later. There was a daily total of 6 tone presentations. The two remaining groups, hearing-no tone and deaf-no tone, were given the same treatment but without the tone presentations. This procedure continued through the 19th day of gestation. Females were not disturbed again until the birth of their litters. At this time, litters were culled to 8 pups, 4 males and 4 females. All experimental litters were fostered out to foster mothers. Litters were weaned at 21 days of age. At 25 days of age, all offspring were tested in a circular open-field for 3 minutes for 3 consecutive days. The following behaviors were recorded per minute: number of sections traversed, frequency of rearing, and amount of time spent grooming. Per session, a cumulative score was obtained for latency to ambulate, amount of defecation, and frequency of grooming.

An analysis of variance on the open-field data revealed effects on latency and ambulation. On latency, the hearing-tone group had the shortest latencies, and the deaf-tone group had the longest latencies. The hearing-no tone and deaf-no tone groups were intermediate to hearing-tone and deaf-tone groups. This difference had disappeared by the second day. On ambulation, there was an effect on the first minute of the first day (fig. 1). The hearing-tone group was significantly different from all other groups which were not different from each other. There

# FIGURE 1

Mean Amount of Ambulation per Minute
on the First Day
for
Hearing-Tone (HT), Hearing-No Tone (HNT),
Deaf-Tone (DT), Deaf-No Tone (DNT),
and
Control (C) Groups


HT
HNT
DT
DNT
C
40
30
20
10
0
MEAN AMOUNT OF AMBULATION
1
2
3
MINUTES

was an inverse relationship between latency and ambulation scores on the first day. The hearing-tone group had the shortest latencies and the highest ambulation scores followed by hearing-no tone, deaf-no tone, deaf-tone, and the controls. This would be expected: the longer the latency, the fewer the number of sections that could be traversed in the first minute.

There were two important findings in this study. First, relative to all other groups, the undisturbed control group was the least active. Simply transporting the maternal cages into the experimental chamber increased the offsprings' ambulation scores above the controls. Second, a tone stimulation effect occurred only if the mother could hear the tone. The other tone stimulation group, deaf-tone, was not significantly different from its control, deaf-no tone. The difference between these two groups and the hearing-tone group indicated that the major component in the tone effect was indirect, mediated by the mother.

# EXPERIMENTS

## Introduction

The pilot study indicated that tone stimulation could produce behavioral differences in the offspring. Furthermore, the ambulation differences were in a direction opposite to that of Morra (1965b). In his study, the sound only group ambulated less than controls. In the pilot study, the hearing-tone group ambulated more than controls. The effect appeared to be indirectly mediated. The sample size of the pilot study, however, was small and litter effects were not considered. Litter membership is of critical importance in any developmental study since behavioral measures within a litter may be correlated (Abbey & Howard, 1973; King, 1969). For this reason, the thesis experiment is a replication of the pilot study but with increased number of mothers per treatment group. By increasing the number of litters per treatment, a small number of individuals can be tested from each litter. This technique decreases the magnitude of litter effects while maintaining a large treatment *n*. Furthermore, each behavioral measure was analysed for litter effects.

There were some modifications made in the experimental design. These included changing the sound stimulus, the addition of another treatment group, and the use of another behavioral test. The sound stimulus was changed from a 2.5 KHz tone to a white noise. Morra (1965b) had used a buzzer as the sound stimulus. It is conceivable that differences in the ambulation scores in the two experiments reflected differences in maternal responsivity to a multiple frequency versus a single frequency stimulus. A hearing low noise group was added as a further control for the deaf noise group. Since deafness of the mothers was not evaluated, the possibility remained that the auditory response was not completely abolished. In either case, the deaf noise group may mimic a hearing low noise group which experiences low intensity noise presentations. A conditioned avoidance task was used in addition to the open-field to test for prenatal effects.

A further explanation is necessary on the addition of a conditioned avoidance task. Studies of the effect of using a neutral CS to induce maternal stress have tested the offspring only in the open-field (Jolley & Adam, 1975; Jolley & Dreesman, 1973; Morra, 1965b). To understand the extent of prenatal stress effects, other behavioral tests are also necessary. Avoidance is one test which has been used in other studies (Joffe, 1969; Joffe, Milkovic, & Levine, 1972; Smith et al., 1975).

Another aspect to the choice of the avoidance task was the possible effects of prenatal stress on the developing pituitary-adrenal axis. Except for a study by Ader and Plaut (1968), there is little data available on the effect of prenatal stress on the pituitary-adrenal axis. There is, however, evidence from early postnatal studies that stress alters the responsiveness of this axis (Levine & Mullins, 1968). Thus, it is conceivable that prenatal stress like postnatal stress affects the later functioning of the pituitary-adrenal axis (Archer & Blackman, 1971). Though it is not possible to verify this assumption without physiological evidence, another approach is the use of behavioral tasks which are influenced by the functioning of the pituitary-adrenal axis.

Two such behavioral tasks are open-field and avoidance behavior. The relationship between open-field behavior and corticosterone levels is ambiguous. There are studies demonstrating a correlation between emotional behavior, as measured in the open-field, and corticosterone levels (Levine, Haltmeyer, Karas, & Denenberg, 1967; Denenberg, 1969). There are also studies showing no such correlation (Ader, 1969; Ader, Friedman, & Grota, 1967; Stern, Erskine, & Levine, 1973). Stern et al. (1973) concluded that though a relationship may occur, it is not obligatory. There are more consistent findings on the role of pituitary-adrenal axis and avoidance behavior. In general, the pituitary-adrenal axis is important during extinction (DiGiusto et al., 1971; Weiss, McEwen, Silva, & Kalkut, 1970). Weiss et al. (1970) attribute the role of pituitary-adrenal function in extinction to its activation in conditions of mild, generalized fear. During acquisition with a clearly signalled noxious event, neural mechanisms will most likely mediate responses rather than the slow acting hormonal system.

Even though acquisition differences have been found in prenatal stress studies (Joffe, 1969; Joffe et al., 1972; Smith et al., 1975), it is not clear what these differences represent. If the pituitary-adrenal axis is involved in both prenatal stress and extinction, then there might be differences in extinction due to prenatal stress. Therefore, in the present study, the effect of prenatal stress on acquisition and extinction was examined.

## Maternal Treatment

The following procedure was used during gestation. Since all the experiments to be presented used offspring resulting from this mating, this procedure will not be presented again.

## Method

### Animals

A total of 153 female hooded rats were obtained from Canadian Breeding Laboratories. Of these, 78 were randomly assigned to the experimental group. The remaining 75 females were assigned to the foster mother pool. The experimental females were individually housed in one holding room, and the foster mothers were individually housed in another room.

### Apparatus for sound presentations

A Grason-Stadler white noise generator was used for the sound presentations. This was connected to an Electronic Switch (Grason-Stadler 829E) with a rise-decay time of 10 msec. and to a Realistic amplifier (SA101). Solid state equipment controlled the sound presentations which occurred at 1 minute intervals with a duration of 3 seconds. There were 3 presentations per session. Animal cages were placed at a distance of 71 cm from the speaker. At this distance, the sound level was 85 db (re $20\mu N/m^2$) for the hearing noise and deaf noise groups, and 65 db (re $20\mu N/m^2$) for the hearing low noise group.

### Procedure

At the beginning of the experiment, the 78 females in the experimental group were randomly assigned to one of 6 conditions: hearing noise (HN), hearing no noise (HNoN), deaf noise (DN), deaf no noise (DNoN), hearing low noise (HLN), or undisturbed controls (C). There were 13 animals per condition.

A month later, all the experimental females including the undisturbed controls were anesthesitized and given a unique ear code. At this time, rats assigned to the deaf groups were deafened. A 16 gauge needle was introduced into the ear canal with the intention of rupturing the tympanic membrane and the ossicular chain. An otoscope was used to ascertain that the membrane had been punctured. Cranioplastic cement powder and its solvent were injected seperately into the ear canal and then mixed together with a probe. Cotton soaked in the plastic cement was used to reinforce the cement plug. The cotton plug was made flush with the ear canal opening rather than protruding into the external meatus. This prevented the rats from dislodging the plug. Two rats died during the operation.

Three days after the operation, mating was begun. Females were divided into small groups. Each group was housed with a breeding male hooded rat in the evening. The following morning, vaginal smears were taken only of the experimental females. Those females who evidenced sperm in the vagina and were in a receptive stage of the estrous cycle were assumed pregnant. Mating was continued for 5 days. After the 5th day, all females were assumed pregnant. For those females who were not classed as pregnant, according to the vaginal smears, the third day of mating was used as the date of pregnancy.

The controls were not disturbed until the birth of their litters. The experimental groups, HN, HNoN, DN, DNoN, and HLN, were not disturbed until the 12th day of gestation. At this time, they were carried in their home cages into the experimental room. Two rats were run at a time, HN with DN, HNoN with DNoN, and one HLN with another HLN. The cages were placed on a table 71 cm away from a speaker. Those animals in the noise presentation groups were exposed to 3 seconds of white noise at 1 minute intervals for a total of 3 presentations. After the last presentation, the rats were returned to the holding room. An hour later, the procedure was repeated for a total of 6 presentations per day. The no noise presentation groups, HNoN and DNoN, received the same procedure but without the noise presentations. This procedure continued through the 19th day of gestation. Females were not disturbed again until the birth of their litters.

During each session, records were made of whether the rat was active--walking, grooming, rearing, or inactive--crouching. Scores were made during noise onset, immediately after noise offset, and between trials. The maternal activity was analysed but the results will not be presented. Activity measures are usually taken to control for possible increases in maternal activity mediating prenatal effects (Jolley & Dreesman, 1973). Except for the HNoN group, there were no significant differences in activity between treatment groups. This indicated that maternal activity was not involved in the prenatal stress effects. Since this measure did not provide information about the prenatal stress effect, further discussion is not necessary.

At birth, the entire litter was sexed and each pup individually weighed. The litter was then culled to 4 males and 4 females. Litters with less than 7 pups were discarded. Fostering of the litter occurred as soon as a foster mother was available, the longest time being 2 days. The total number of litters in each condition was as follows: HN, HNoN, HLN, and C had 5 litters each; DN and DNoN had 3 litters each. Litters were weaned at 21 days of age. At this time, all litter members were housed together. After the open-field test, there were only 4 animals per litter. At 35 days of age, litter members were re-housed by sex. This condition was maintained for the remainder of the experiments.

## EXPERIMENT 1

Previous studies of the effect of a neutral stimulus as a maternal stressor have used the open-field to test the offspring. In one study (Morra, 1965b), the offspring were more emotional; in the other studies (Jolley & Adam, 1975; Jolley & Dreesman, 1973), the offspring were less emotional. In the present study, the offspring were tested in the open-field to examine possible direct effects of sound stimulation. If the deaf noise group is significantly different from both its controls, deaf no noise and hearing low noise, then part of the effect of sound stimulation is a direct effect on the fetus. Hence, the difference between light (Jolley & Adam, 1975; Jolley & Dreesman, 1973) and sound (Morra, 1965b) as neutral stimuli would be a difference in mediation.

### Method

#### Animals

At 25 days of age, 2 males and 2 females were randomly picked from each litter for a total of 104 animals. There were 20 animals in each of the following conditions: HN, HNoN, HLN, and C; 12 animals each in DN and DNoN.

#### Apparatus

A circular open-field modelled after Broadhurst (1960) was used. It was 83 cm in diameter with 31.75 cm high white sides. Illumination was provided by a 60 watt light bulb placed 48.26 cm above the center circle of the open-field. This provided a floor illumination of 91.3 millilamberts (290.33 cd/$m^2$) as measured by a

Macbeth illuminometer (6800). A Grason-Stadler white noise generator was on for each session and provided a 70 db (re 20$\mu$N/m$^2$) background noise when measured in the open-field. A push button board was used to record latency to ambulate, number of sections traversed, frequency of rearing, and time spent grooming (grooming duration). These last three scores were accumulated on a printing counter which printed scores every minute. Latency to ambulate and frequency of grooming accumulated on counters. These last two measures and amount of defecation were recorded at the end of each session. Each session lasted for 3 minutes.

### Procedure

Rats were individually introduced into the open-field and placed in the center circle. As soon as the rat was released, the latency counter started and was stopped when the rat moved out of the center circle. A section was considered crossed only when the rat had placed all four feet in it. At the end of the 3 minute trial, the rat was removed, a bolus count taken, and the field wiped clean with a weak detergent. Trials were random across days with each rat being tested for 3 consecutive days. Animals were weighed on the last day of testing.

## Results

An unweighted means analysis of variance was performed on the data since there were an unequal number of litters between treatments. The analysis was a Treatment (6) x Days (3) with Litters nested within Treatment and Subjects being nested within Treatment and Litters. Sex was not analysed since it was balanced within litters, and it would have reduced the cell n. There were a total of six analyses, one for each measure. Planned comparisons (LSD) were also performed on the data. The latter approach was taken since the experiment was designed with critical comparisons. These were HN to HNoN, DN to DNoN, DN to HLN, DN to HN, and the controls compared to all groups. In obtaining the proper within mean square, the following procedure was used. An F ratio was obtained of Litters within Treatments and Subjects within Litters/Treatments. If the p value of this F ratio was greater than .20, then the two within sum of squares were pooled, and a mean square obtained for Subjects within Treatments. A p value of .20 is a fairly conservative test (Winer, 1971, p. 379). If the obtained F ratio was less than .20, the within mean square for Litters within Treatments was used.

### Ambulation

In the analysis of variance of ambulation (Table 1), there was only a days effect ($F$ (2,40) = 55.75, $p < .01$). Ambulation scores for all goups decreased across days (fig. 1).

Planned comparisons were performed on the treatment effect and on the Treatment x Days interaction. The mean square for Subjects within Treatments was used in the main effect of treatment ($F$ (20,78) = 1.27, $p > .2$). The mean square for Litters within Treatment x Days was used for Treatment x Days comparisons ($F$ (40,156) = 2.38, $p < .01$).

There was a treatment effect on ambulation. HN

## TABLE 1

## An Unweighted Means Analysis of Variance on Ambulation

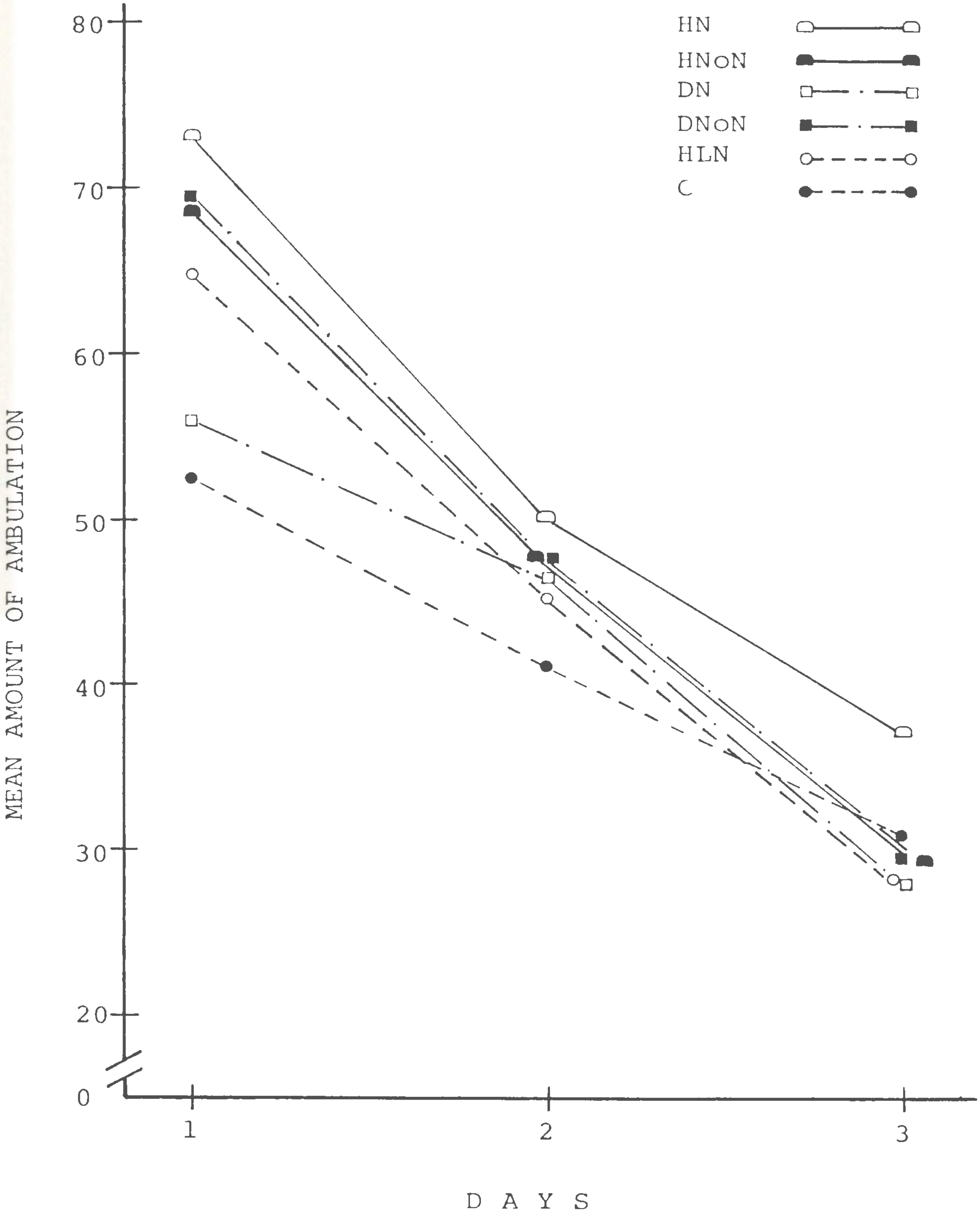
| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 4572.85 | 5 | 914.57 | 2.00 |
| Subjects/T[a] | 44893.76 | 98 | 458.10 | |
| Days (D) | 55661.96 | 2 | 27830.98 | 55.75** |
| D x T | 2762.03 | 10 | 276.20 | .55 |
| D x L/T | 19969.91 | 40 | 499.25 | 2.38** |
| D x S/LT | 32787.17 | 156 | 210.17 | |

[a] L/T $F$ (20,78) = 1.27, $p > .2$

**$p < .01$

FIGURE 2

Mean Amount of Ambulation across Days



HN
HNoN
DN
DNoN
HLN
C
80
70
60
50
40
30
20
0
MEAN AMOUNT OF AMBULATION
1
2
3
DAYS

ambulated significantly more than either DN ($p < .05$) or C ($p < .01$). Though all groups ambulated more than the undisturbed controls, none of the other comparisons were significant.

The mean ambulation per day for each treatment is presented in figure 2. The difference between HN and C (20.55) was just short of significance. The $CR_{LSD}$ must be 20.88 or greater for $p < .05$. There were no significant differences on Day 2 or Day 3. Since there were no significant differences on Day 2 or Day 3, the overall treatment effect must be due to the high ambulation scores of HN on Day 1 (fig. 2).

Grooming Duration

In the analysis of variance on grooming (Table 2), there was a significant treatment effect ($F$ (5,98) = 3.63, $p < .01$) and a significant Treatment x Days interaction ($F$ (10,196) = 2.01, $p < .05$).

Planned comparisons were performed on the main treatment effect and on the Treatment x Days interaction. The mean square for Subjects within Treatments ($F$ (20,78) = 1.13, $p > .2$) was used for comparisons of the treatment effect. The mean square for Subjects within Treatment x Days ($F$ (40,156) = .84, $p > .2$) was used for the comparisons within days.

The treatment effect was confined to the DN condition which groomed less than all of its comparison groups: HN ($p < .05$), DNoN ($p < .01$), HLN ($p < .05$), and C ($p < .01$).

The mean grooming duration per day for each treatment

TABLE 2

An Unweighted Means Analysis of Variance on Grooming Duration

| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 8966.68 | 5 | 1793.34 | 3.63** |
| Subjects/T[a] | 48387.35 | 98 | 493.75 | |
| Days (D) | 210.89 | 2 | 105.45 | .52 |
| D x T | 4101.51 | 10 | 410.15 | 2.01 |
| D x S/T[b] | 39948.54 | 196 | 203.82 | |

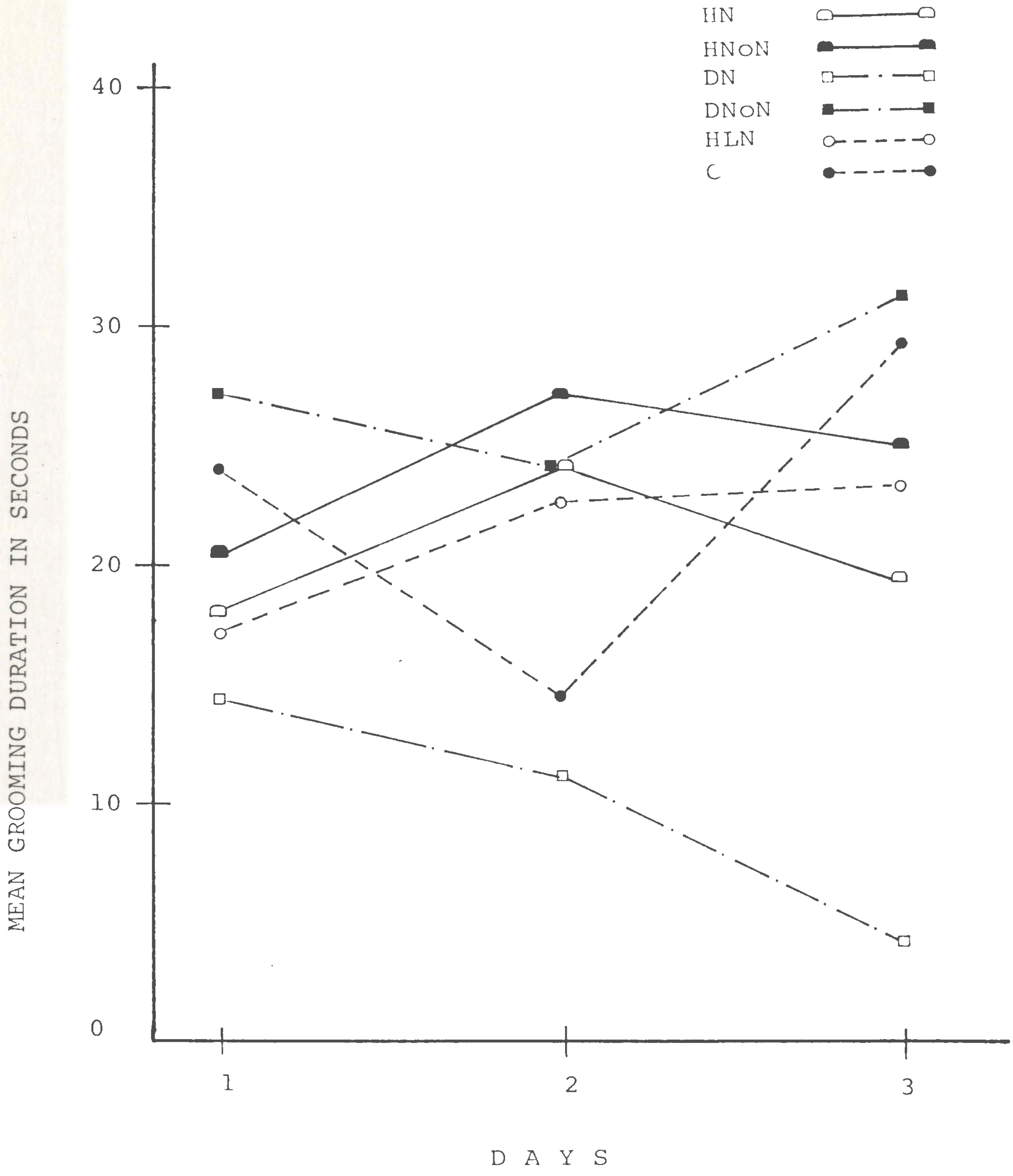
[a] L/T $\underline{F}$ (20,78) = 1.13, $\underline{p} > .2$

[b] D x L/T $\underline{F}$ (40,156) = .84, $\underline{p} > .2$

**$\underline{p} < .01$

FIGURE 3

Mean Grooming Duration across Days


HN
HNoN
DN
DNoN
HLN
C
40
30
20
10
0
MEAN GROOMING DURATION IN SECONDS
1
2
3
DAYS

is presented in figure 3. Even on Day 1, the DN group was grooming less than any other group though DN was only significantly different from DNoN ($p < .05$). None of the other comparisons were significant. The decreased grooming of the DN group became more pronounced on Days 2 and 3. On Day 2, DN was grooming significantly less than DNoN ($p < .05$), HLN ($p < .05$), and HN ($p < .05$). On Day 3, DN was significantly different from HN ($p < .05$), DNoN ($p < .01$), HLN ($p < .01$), and C ($p < .01$).

Grooming frequency

In the analysis of variance on grooming frequency (Table 3), there was a significant days effect ($F$ (2,196) = 7.95, $p < .01$). The number of instances of grooming decreased across days even though the duration (fig. 3) tended to increase.

Planned comparisons were performed on the main treatment effect and on the Treatment x Days interaction. The mean square for Litters within Treatments ($F$ (20,78) = 1.34, $p > .2$) was used for comparisons of the treatment effect. The mean square for Subjects within Treatments x Days ($F$ (40,156) = .84, $p > .2$) was used for comparisons within days.

The decreased grooming duration of DN was reflected in a decreased frequency of grooming. On the main treatment effect, DN differed significantly from DNoN ($p < .01$) but not from HLN ($p > .05$).

The mean frequency of grooming per day for each treatment is presented in figure 4. On Day 1, DN was

# TABLE 3

## An Unweighted Means Analysis of Variance on Grooming Frequency

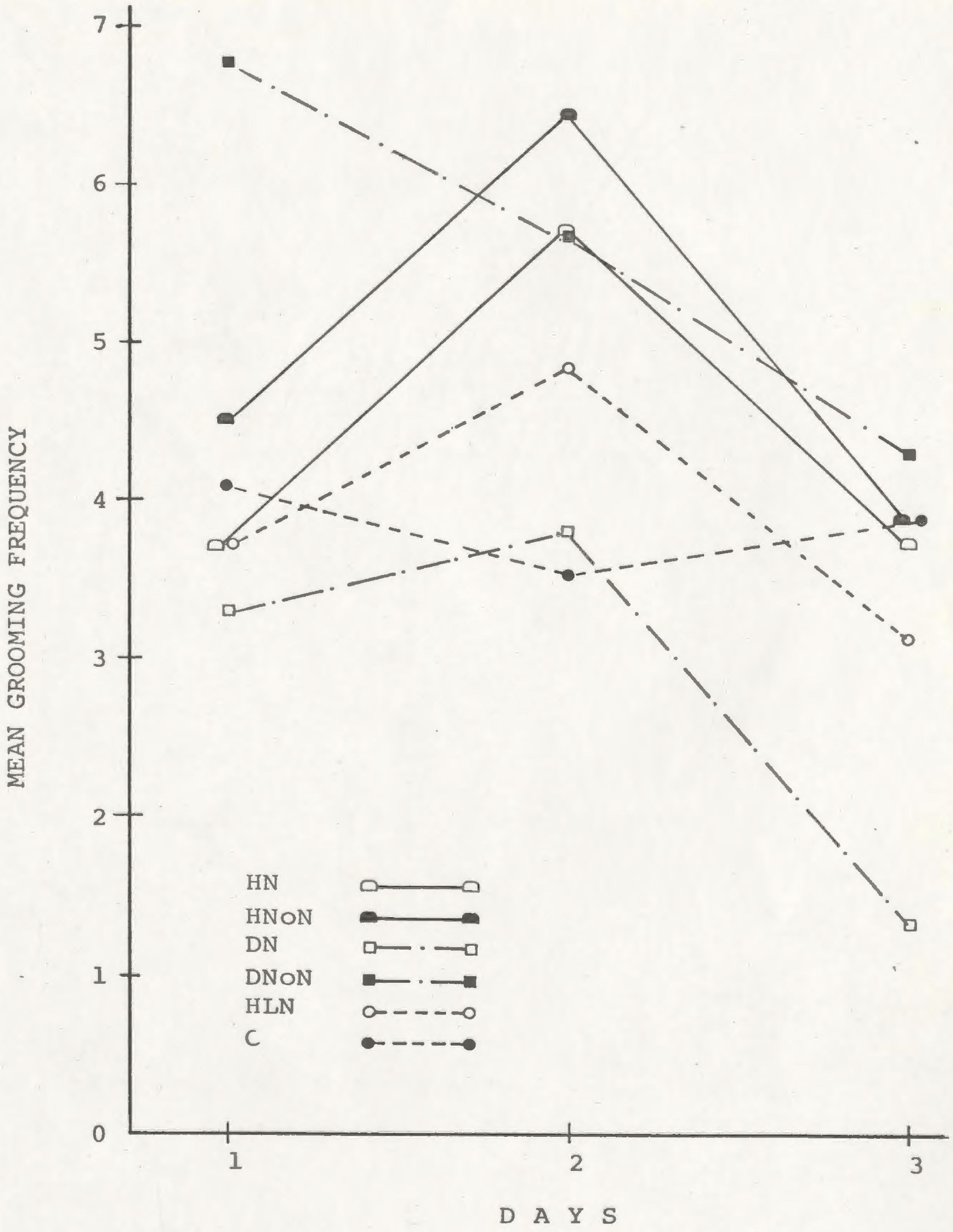
| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 269.21 | 5 | 53.84 | 2.32 |
| Litters/T | 463.89 | 20 | 23.19 | 1.34 |
| Subjects/LT | 1343.50 | 78 | 17.22 | |
| Days (D) | 116.88 | 2 | 58.44 | 7.95** |
| D x T | 105.01 | 10 | 10.50 | 1.42 |
| D x S/T[a] | 1440.31 | 196 | 7.35 | |

[a] D x L/T $\underline{F}$ (40,156) = .83, $\underline{p} > .2$

**$\underline{p} < .01$

FIGURE 4

Mean Grooming Frequency across Days


MEAN GROOMING FREQUENCY
0
1
2
3
4
5
6
7
1
2
3
DAYS
HN
HNoN
DN
DNoN
HLN
C

significantly different from DNoN ($p < .01$). DNoN also differed from C ($p < .05$). On Day 2, DN differed from DNoN ($p < .05$) and from HN ($p < .05$). On Day 3, DN differed from DNoN ($p < .01$), HN ($p < .05$), and C ($p < .05$).

## Defecation

In the analysis of variance on defecation (Table 4), there were no significant effects.

Planned comparisons were performed on the main treatment effect and on the Treatment x Days interaction. The mean square for Litters within Treatments ($F$ (20,78) = 1.69, $p > .2$) was used for comparisons of the treatment effect. The mean square for Litters within Treatments x Days ($F$ (40,156) = 1.47, $p > .2$) was used for comparisons within days.

There was no main treatment effect.

The mean amount of defecation per day for each treatment is presented in figure 5. The only significant difference occurred on Day 3. HN defecated significantly more than HNoN ($p < .05$) and DN ($p < .05$). DN also defecated less than HLN ($p < .05$) but was not significantly different from DNoN ($p > .05$).

## Latency

In the analysis of variance on latency (Table 5), there was a significant days effect ($F$ (2,40) = 17.75, $p < .01$). Latency for all groups decreased across days.

Planned comparisons were performed on the main treatment effect and on the Treatment x Days interaction. However, none of these comparisons produced significant

## TABLE 4

## An Unweighted Means Analysis of Variance on Defecation

| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 21.23 | 5 | 4.25 | 1.03 |
| Litters / T | 82.39 | 20 | 4.12 | 1.69 |
| Subjects/LT | 190.42 | 78 | 2.44 | |
| Days (D) | 10.33 | 2 | 5.17 | 2.25 |
| D x T | 47.67 | 10 | 4.77 | 2.07 |
| D x L/T | 91.84 | 40 | 2.30 | 1.47 |
| D x S/LT | 243.83 | 156 | 1.56 | |

FIGURE 5

Mean Amount of Defecation across Days

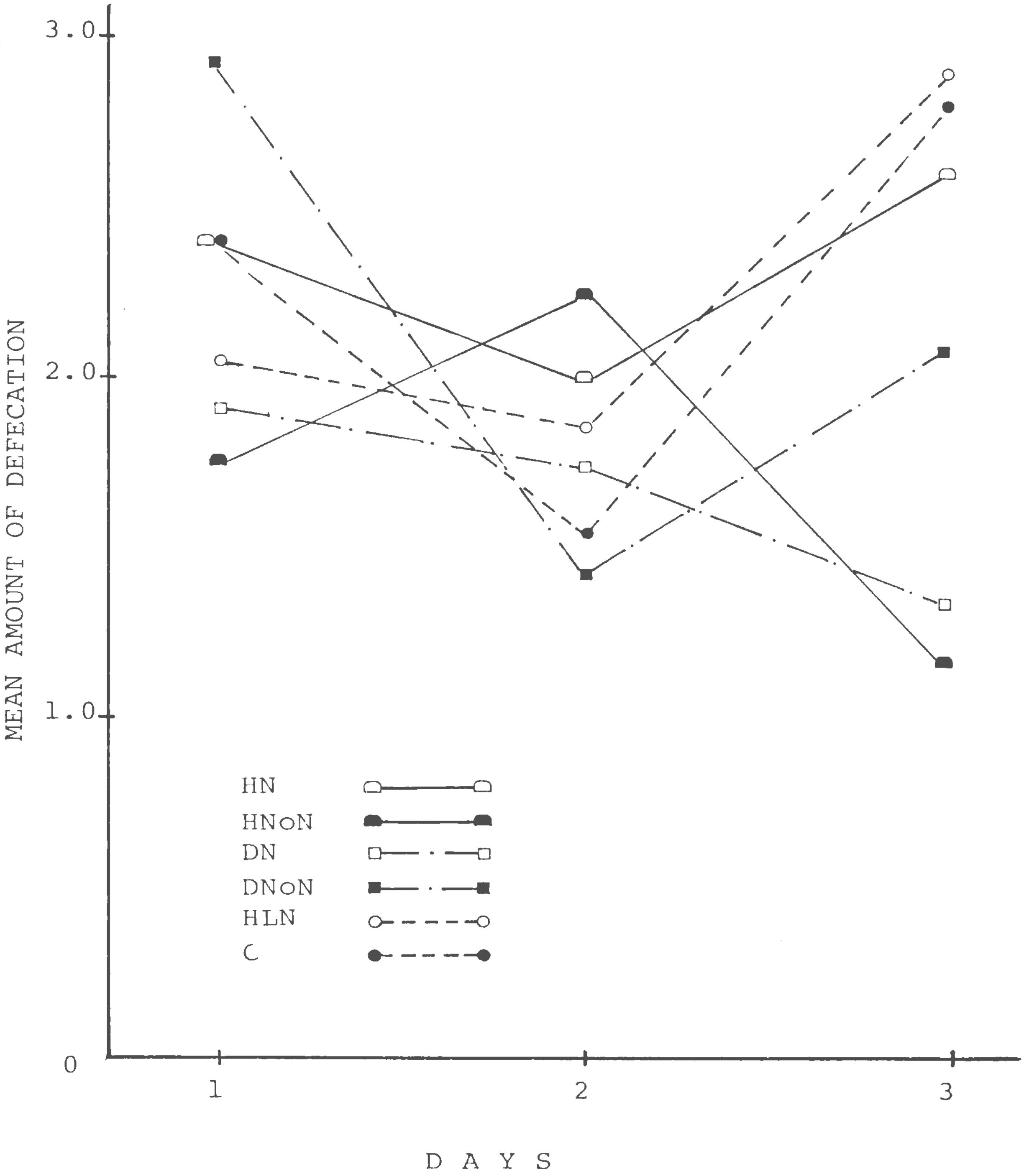

MEAN AMOUNT OF DEFECATION
3.0
2.0
1.0
0
1
2
3
D A Y S
HN
HNoN
DN
DNoN
HLN
C

TABLE 5

An Unweighted Means Analysis of Variance on Latency

| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 5.28 | 5 | 1.06 | .97 |
| Subjects/T[a] | 106.47 | 98 | 1.09 | |
| Days (D) | 39.75 | 2 | 19.88 | 17.75** |
| D x T | 8.45 | 10 | .85 | .76 |
| D x L/T | 44.93 | 40 | 1.12 | 1.42 |
| D x S/LT | 123.50 | 156 | .79 | |

[a] L/T *F* (20,78) = .61, $p > .2$

**$p < .01$

## TABLE 6

## An Unweighted Means Analysis of Variance on Rearing

| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 546.06 | 5 | 109.21 | .77 |
| Litters/ T | 2847.50 | 20 | 142.38 | 1.97 |
| Subjects/LT | 5631.33 | 78 | 72.20 | |
| Days (D) | 2680.10 | 2 | 1340.05 | 32.78** |
| D x T | 143.73 | 10 | 14.37 | .35 |
| D x L/T | 1635.07 | 40 | 40.88 | 1.36 |
| D x S/LT | 4672.17 | 156 | 29.95 | |

**$p < .01$

differences.

Rearing

In the analysis of variance on rearing (Table 6), there was a significant days effect ($F$ (2,40) = 32.78, $p < .01$). The amount of rearing for all groups decreased across days.

Planned comparisons were performed on the main treatment effect and on the Treatment x Days interaction. However, none of these comparisons produced significant differences.

## Discussion

Of the six behavioral measures taken in the open-field, three measures: grooming frequency, grooming duration, and ambulation yielded significant treatment effects. There was a treatment effect on defecation but this was confined to the third day.

The defecation data presented an ambiguous picture. There was no overall treatment effect, and there was no consistent pattern of defecation across days. Usually defecation decreases across days (Archer, 1973; Ivinskis, 1968). Furthermore, if defecation is compared to ambulation (see figs. 2 & 5), the differences in defecation on the third day were not reflected in any differences in ambulation. Though there is conflicting evidence (Archer, 1973), defecation is usually inversely correlated with ambulation (Denenberg, 1969). However, as is readily apparent from comparing ambulation scores to defecation scores, there does not seem to be any such relationship between these two measures. Because of the lack of an overall treatment effect and the inconsistent patterns between groups across days, the weak treatment effect on the third day is unexplainable and is considered to be of little importance.

The effect in grooming frequency supported the grooming duration data. Even though there was a days effect, the frequency scores remained fairly stable across days. When this data were compared with the grooming duration data, the increase in grooming across days reflected an increase in time spent grooming rather than in an increase in the frequency of its occurrence.

The treatment effect on grooming duration was due solely to the DN group which differed from its two control groups: DNoN and HLN. Furthermore, in respect to all treatment groups, the DN group spent less time grooming. This effect became more pronounced across days. Whereas the other treatment groups showed a tendency to increase grooming across days, the DN group showed a slight decreased across days.

The decrease in grooming scores of the DN group was not expected. Though there are different interpretations of grooming (Fentress, 1968), there is general agreement on the pattern of grooming in the rat. Grooming increases across days (Archer, 1973; Doyle & Yule, 1959) and is usually associated with a decrease in activity across days (Bolles, 1963; Hughes, 1968; Prescott, 1970). This particular pattern of an increase in grooming and a decrease in activity occurred in all the treatment groups except the DN group. Since there were no differences in the HLN and DNoN groups, the dramatic effect of noise stimulation in the DN group would appear to be the result of a direct effect of noise stimulation on the fetus. For an effect of this magnitude, however, one would expect some indication from the pilot work. In the previously reported pilot study, there was no such treatment effect. The difference between studies is a reflection of one of the problems in using grooming: it has low reliability (Ivinskis, 1968). So even though there was a grooming effect, it is not clear what interpretation it should be given nor how important an effect it is.

As expected from the pilot study, there was a treatment effect on ambulation. HN ambulated significantly more than DN and C. This effect appeared to be due to marked differences on the first day since there were no noticeable differences in ambulation scores on the second or third day. In accordance with other prenatal studies (Archer & Blackman, 1971), the increased ambulation in the experimental conditions relative to the controls was interpreted as indicating a decreased emotionality. This decrease in emotionality contrasts with Morra's study (1965b) in which sound stimulation increased emotionality. In the present study, however, the prenatal effect is not confounded with a postnatal effect since all offspring were fostered out to controls.

There are two components to the treatment effect: an effect of noise stimulation and an effect of introduction into the experimental chamber. A noise stimulation effect occurred only in the hearing condition. DN while differing from the HN group was not significantly different from DNoN or HLN, the two controls for the DN group. The lack of differences between these three groups mitigates against an interpretation of a direct effect of noise stimulation. For a noise stimulation effect to occur, the mother must be able to perceive and respond to the noise. Hence, the different effect of light and sound on emotionality (Jolley & Adam, 1975; Jolley & Dreesman, 1973; Morra, 1965b) is not due to a direct effect of sound.

The second component of the treatment effect was the introduction into the experimental chamber. Of all the treatment groups, the controls had the lowest ambulation scores. The only consistent difference between the controls and the experimental groups was that all experimental groups were introduced into the experimental chamber. Noise stimulation was not necessary for this effect. The fact that exposure to the experimental chamber altered ambulation scores illustrates that any disturbance of the maternal environment affects the offspring. This raises the question as to whether the degree of disturbance in the maternal environment is a critical factor in prenatal stress effects. Disturbance of the maternal environment is not the same as that proposed by Hutchings and Gibbon (1970) in which it was suggested that prenatal effects are the result of disrupting nesting behavior. The studies showing effects of different stress intensities do not support this position (Archer & Blackman, 1971). Nor is it supported in the present study since the mother was never removed from the home cage. Rather the interest is in the amount of stimulation the mother is subjected to.

There was a procedural difference between the present study and Morra's. The mothers in the present study were never removed from their home cages. The same procedure was used in Jolley and Adam (1975), and Jolley and Dreesman (1973). Even though a light was the neutral stimulus in the work of Jolley, the offspring were less emotional than controls. In Morra's study, the mothers were removed from their home cages, placed in a novel environment, and given noise presentations. Introduction into a novel environment does elicit a stress response, an increase in corticosterone levels (Bassett, Cairncross, & King, 1973). The question is whether a novel stimulus in a novel environment results in a different or greater stress response than presentations of a novel stimulus in a familiar environment. If the stress response is greater the offspring may be more emotional. This is similar to early stimulation effects in which an increase in stimulation results in more emotional offspring (Henderson, 1968). Therefore, the difference in the study of Morra and Jolley may be the result of the procedural difference.

In conclusion, the open-field yielded effects on two behavioral measures: ambulation and grooming duration. Of the two measures, grooming behavior was questionable in terms of reliability. Ambulation on the other hand, indicated that the predominant effect of noise stimulation during gestation is indirectly mediated. However, the experimental procedure itself contributed to the effect as all groups showed a slight increase in activity over the controls.

## EXPERIMENT 2

A shuttle box avoidance task was used to extend the findings of the effect of a neutral stimulus as a maternal stressor. Previous studies have been confined to tests in the open-field (Jolley & Adam, 1975; Jolley & Dreesman, 1973; Morra, 1965b). Prenatal stress has been found to affect avoidance behavior (Joffe, 1969; Joffe et al., 1972; Smith et al., 1975). Of particular interest, in the present study, was the possible effect of prenatal stress on extinction behavior since this measure is sensitive to alterations in the pituitary-adrenal axis (DiGuisto et al, 1971; Weiss et al., 1970). If prenatal stress affects this system, then there might be differences on extinction.

### Method

#### Animals

At 41 days of age, one female was chosen from each litter for a total of 5 animals each in HN, HNoN, HLN, and C conditions; and 3 animals each in DN and DNoN. The use of females for the avoidance training was based on previous prenatal studies. In these studies, only the females showed a treatment effect (Joffe et al., 1972; Smith et al., 1975).

#### Apparatus

The shuttle box was 15.88 cm wide, 74.93 cm long with 60.96 cm white sides. A hurdle 3.81 cm high divided the compartment in half with 24 stainless steel rods equally spaced per side. These rods were wired for neon scrambled shock. A photo cell was positioned 1.91 cm above the hurdle.

The CS was a 75 db (re $20 \mu N/m^2$) white noise provided by a Grason-Stadler white noise generator. The UCS was .35 ma scrambled shock provided by a Lafeyette master shocker. Solid state equipment controlled CS and UCS presentations. The CS preceded the UCS by 8 seconds. The UCS was on for 10 seconds at which time both the CS and UCS terminated. The intertrial interval was 60 seconds.

## Procedure

Animals were individually introduced into the chamber and given 3 minutes of adaptation. Avoidance training was then begun. CS onset started a cumulative counter printer. As soon as the photo cell beam was broken, the latency counter stopped, and the latency score was printed. The termination of the CS did not terminate UCS onset. Problems were encountered in the use of the photo cell beam. Some rats showed a tendency to cut the photo cell beam with a nose poke rather than shuttling from the to-be-shocked side to the safe side. In order to circumvent this problem, the UCS always occurred. The avoidance procedure was as follows. A response during the 8 second CS onset terminated the CS but not the UCS. This was counted as an avoidance response. A response that occurred during the CS-UCS period terminated the CS but not the UCS. This was counted as an escape response. There were 15 trials a day until 12 out of 15 avoidances occurred (80%) or until 315 trials. After the 80% criteria was attained, extinction trials were begun the next day. Fifteen extinction trials were given a day for a total of 75 trials.

## Results

No analysis was performed on the data. After 315 trials, only 2 of 5 control animals had attained the 80% acquisition criteria. A similar pattern occurred across the experimental conditions. Of the 9 animals which acquired the response, at least 5 did not respond for at least half of the extinction trials. With such low acquisition rates within groups and such a poor baseline group (the controls), there was no reason to analyse essentially similar data points.

## Discussion

The shuttle box has been used in other prenatal stress studies. Furthermore, performance on this task particularly during extinction is sensitive to alterations in the pituitary-adrenal axis. Unfortunately, the results of this study were less than clear. Though the parameters of this experiment were similar to other studies, all of the treatment groups had very poor performance scores. This may be a function of the task itself. The shuttle box is a relatively difficult task for a rat to acquire, and there is always a certain proportion of subjects which do not learn the response (Brush, 1966). The small sample size may only have augmented this effect. Furthermore, some modification of the apparatus may have facilitated acquisition: a buzzer seems to be more effective than a noise as a CS (Myers, 1959), and the use of a guillotine door appears to facilitate the acquisition of a running response (Fantino, 1973).

## EXPERIMENT 3

Since the shuttle box data were inconclusive, a third study was conducted to determine if using a different task a neutral stimulus as a maternal stressor affects avoidance behavior. A one-way avoidance task was chosen. The particular apparatus was a circular avoidance chamber. A circular avoidance chamber eliminates handling of the animal between trials. Handling may be one factor which contributes to the rapid acquisition of a one-way task since it disrupts freezing behavior (Stewart & Anisman, 1970; Theios, Lynch, & Lowe, 1966). If the number of trials before acquisition occurs is lengthened using this procedure, then possible differences in acquisition may appear. Extinction behavior was also examined.

### Method

#### Animals

At 145-160 days of age, female rats which had not been previously tested in the shuttle box were run in the circular avoidance task. There were 5 females each in HN, HLN, and C conditions; 4 from the HNoN condition; and 3 each in DN and DNoN conditions. In an effort to increase the sample size, females previously tested on the shuttle box task were given 5 CS exposures. Their response latencies, however, indicated a prior testing effect. Thus these animals were not given further avoidance training.

#### Apparatus

The circular one-way avoidance chamber was similar to one used by Stewart and Anisman (1970). It had an inner diameter of 27.94 cm and an alley width of 9.53 cm. The sides were 23.18 cm high and painted flat black. The apparatus was 25.4 cm off the floor. The runway was divided into 4 quadrants with 33 brass rods per quadrant. Each quadrant was separated from the next by a black partition 26.04 cm high. When the partition was lowered through the bars, a 1.91 cm hurdle was formed and allowed the rat access to the next quadrant. A 12 volt light was positioned in each quadrant 1.25 cm from the top of the apparatus and 2.54 cm in front of each partition. A speaker was positioned in the inner circle.

The CS was a compound of light and tone onset. The tone was an 8 KHz tone provided by a Hewlett Packard frequency generator (3310B) and a Harmon Kardon amplifier. This produced a 79 db (re 20$\mu$N/m$^2$) tone in the runway. There was a 12 volt light in each quadrant. The UCS was a .8 ma scrambled shock provided by a Grason-Stadler shock generator (E1064).

Solid state equipment controlled CS and UCS presentations. The CS preceded the UCS by 7 seconds. The UCS was on for 30 seconds at which time both the CS and UCS terminated. The intertrial interval was 30 seconds. During extinction, only the CS was presented for 37 seconds. The intertrial interval remained 30 seconds.

### Procedure

Animals were individually introduced into the chamber and given 5 CS pre-exposure trials. This consisted of the compound CS with access to the next quadrant. A latency score was recorded for each presentation. After all animals had received the 5 pre-exposure trials, 25 acquisition trials were given on the same day. The procedure was as follows. CS onset started a latency counter, and the quadrant partition was manually dropped to allow access to the next quadrant. As soon as the hurdle was crossed, a push button stopped the latency counter and the time was recorded. A response prior to UCS onset terminated the CS and was counted as an avoidance. A response after UCS onset terminated both the CS and UCS. Animals ran in a counter-clockwise direction. The next day acquisition trials continued until 8 out of 10 avoidance responses were made or until 80 trials. This was followed immediately by 40 extinction trials. Extinction continued to 2 days, 80 trials per day. Criteria for extinction was failure to avoid on 8 out of 10 trials or until 200 trials.

## Results

The 5 CS pre-exposure latencies for animals with prior shuttle box training were compared to the latencies of their naive litter mates by use of a matched *t* test. There were significant differences in HLN ($t$ (8) = 2.77, $p < .05$), DNoN ($t$ (4) = 4.37, $p < .05$), and HN ($t$ (8) = 5.27, $p < .01$). In all three instances, the naive litter mates had shorter response latencies than animals with prior shuttle box experience.

An unweighted analysis of variance was performed on the CS pre-exposure latencies of animals given avoidance training (Table 7). The design was a Treatments x Trials with Subjects nested within Treatments. There was no significant treatment effect (F (5,19) = 2.31, $p > .10$) but there was a significant trials effect (F (4,76) = 2.83, $p < .05$). Latency of response decreased across trials (fig. 6). A planned comparison was performed on the treatment effect. HN, DNoN, and HLN were significantly different from the controls ($p < .05$). All three groups had shorter response latencies relative to the controls (fig. 6). No other comparisons were significant.

Two unweighted means analyses of variance were performed on the acquisition data. The first analysis was on the number of avoidances on the first day. Only the last 15 trials of the first day were analysed. During the first 10 trials some animals continued to respond to the CS without experiencing the UCS. By the tenth trial, however, every rat had received at least one shock. There was a significant treatment effect (F (5, 19) = 6.09, $p < .01$) (Table 8). This effect was analysed further by planned comparisons DN was significantly different from HN ($p < .01$), DNoN ($p < .01$), and C ($p < .01$). HLN was not significantly different from DN but was different from C ($p < .01$). As seen in figure 7, both DN and HLN had a high number of avoidances compared to the other groups. The differences on the first day were not reflected in any differences in the total number of trials to reach acquisition (F (5,19) = 1.18, $p > .10$) (Table 9).

Animals which did not attain the 80% acquisition

# TABLE 7

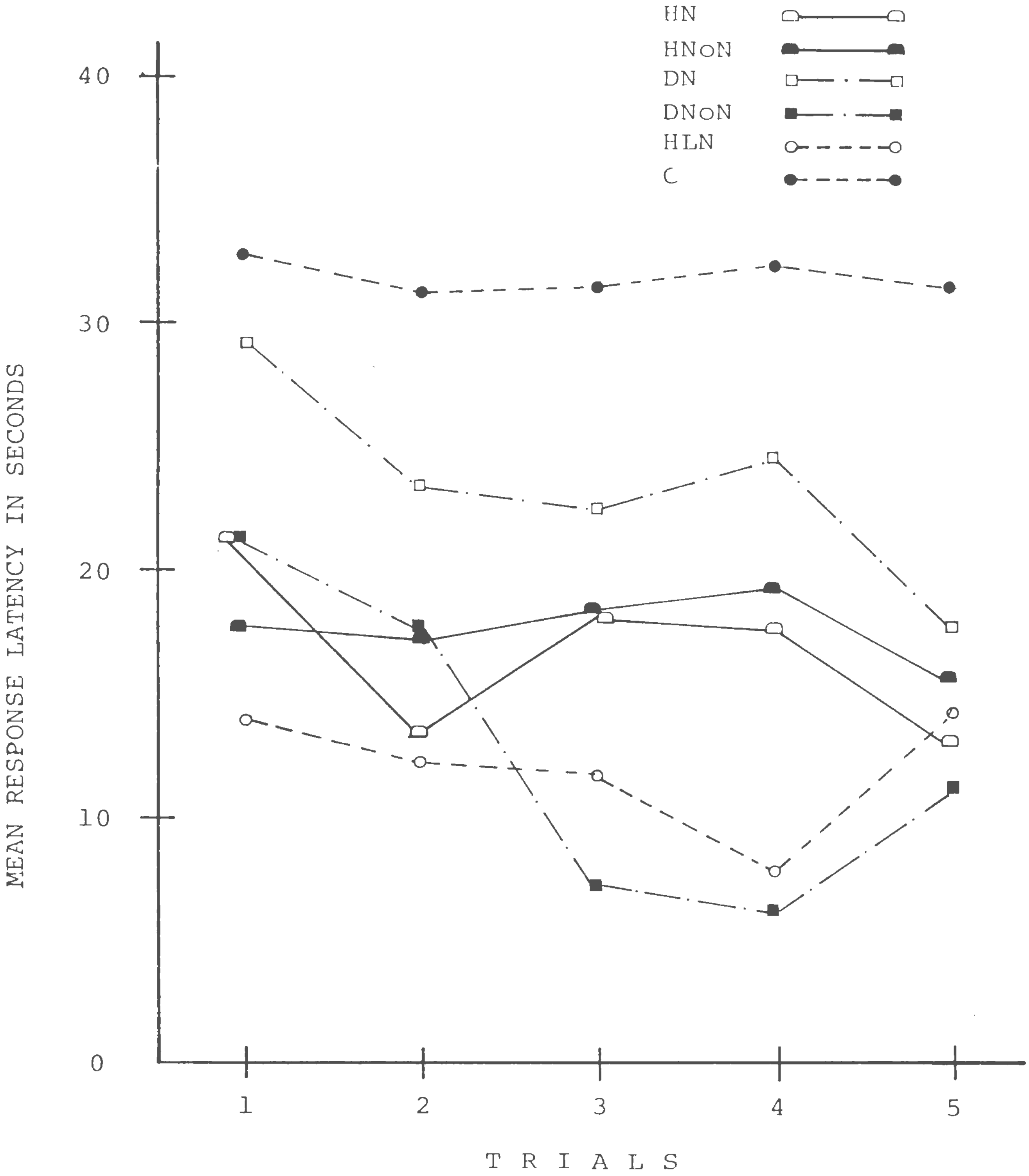
# An Unweighted Means Analysis of Variance
on
Latency of Response to CS Pre-exposures

| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 5483.10 | 5 | 1096.62 | 2.31 |
| Subjects/T | 9002.41 | 19 | 473.81 | |
| Trials | 436.41 | 4 | 109.10 | 2.83* |
| Trials x T | 846.10 | 20 | 42.30 | 1.10 |
| Trials x S/T | 2924.68 | 76 | 38.48 | |

*$p < .05$

FIGURE 6

Mean CS Pre-exposure Response Latencies during the Five Presentations


HN
HNoN
DN
DNoN
HLN
C
40
30
20
10
0
MEAN RESPONSE LATENCY IN SECONDS
1
2
3
4
5
TRIALS

## TABLE 8

### An Unweighted Means Analysis of Variance on Number of Avoidances on the First Day[a]

| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 161.22 | 5 | 32.24 | 6.09** |
| Subjects/T | 100.53 | 19 | 5.29 | |

[a] only the last 15 trials on the first day were analysed

**$p < .01$

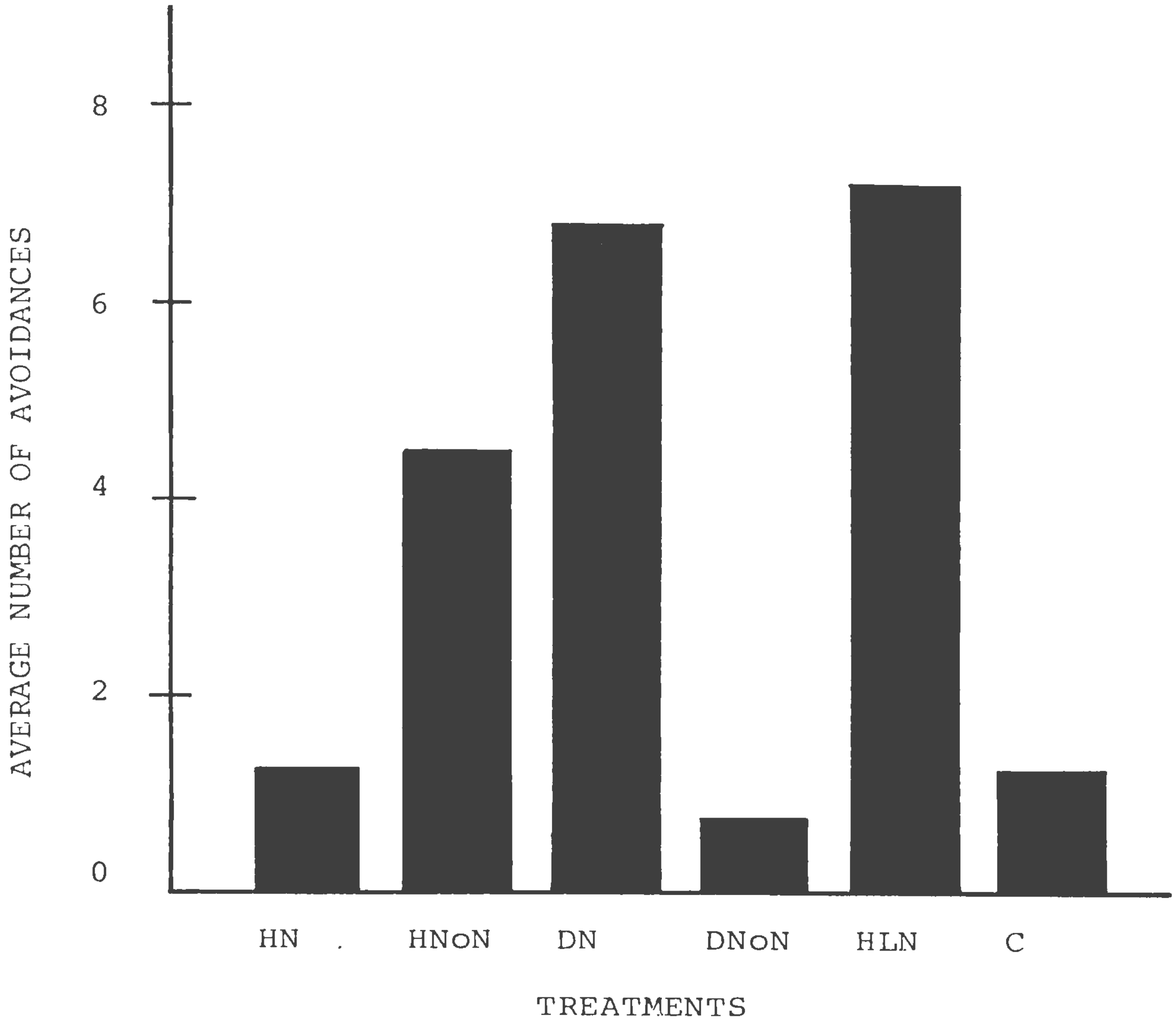
## TABLE 9

### An Unweighted Means Analysis of Variance on Number of Trials to Acquisition

| | SS | df | MS | F |
|---|---|---|---|---|
| Treatment (T) | 2916.55 | 5 | 583.31 | 1.18 |
| Subjects/T | 9383.82 | 19 | 493.88 | |

FIGURE 7

Average Number of Avoidances for the Last Fifteen Trials of the First Day of Training


AVERAGE NUMBER OF AVOIDANCES
8
6
4
2
0
HN
HNoN
DN
DNoN
HLN
C
TREATMENTS

criterion were excluded from the extinction analysis. There was one animal each in HN, HNoN, and DNoN. A Fisher exact test was used to analyse the extinction data. Animals were either classified as extinguishing within 200 trials or as still responding. The only significant effect occurred between HN and C (p = .04). Four animals in the HN group had not extinguished in 200 trials compared to four out of five animals in the control condition.

## Discussion

There were three measures on the one-way avoidance task which yielded significant results: latency of response to the CS alone, number of avoidances on the first training day, and number of avoidance trials to reach the extinction criteria.

There were two aspects to the CS pre-exposure presentations: the effect of prior shuttle box experience and the effect of prenatal treatment. In three treatment groups HN, HLN, and DNoN, prior shuttle box training significantly altered response latencies to the CS alone. In all three groups, prior training on the shuttle box significantly increased response latencies compared to naive litter mates. Though a prior experience effect was not expected, it precluded including any animals with shuttle box training in the one-way avoidance task. Their avoidance performance might have differed from naive litter mates.

A prenatal treatment effect appeared in the response latencies of the naive animals. HN, HLN, and DNoN had response latencies which were significantly shorter than the control group. Even though HNoN and DN were not significantly different from the controls, all groups had shorter response latencies than the controls. This pattern of results, the controls being the least active, is similar to the ambulation scores in the open-field. The controls ambulated less than any other group. Thus some aspect of the experimental treatment increased ambulation scores in the open-field and decreased the latency of response to a neutral stimulus relative to the controls. Noise stimulation per se is not crucial for this effect. Furthermore, since the open-field ambulation scores are used as a measure of reactivity, the latency of response to the CS may also be a measure of emotional reactivity.

The idea that the latency score may reflect reactivity was further supported by observations of animals with long latency scores. A long latency response was characterized by little motor movement, i.e. freezing. Freezing has been used as an index of fear in the open-field (Doyle & Yule, 1959) and in conditions previously associated with shock (Blanchard & Blanchard, 1969). Thus it would appear that animals with long latencies were more fearful of the conditions than animals with short latencies. A similar interpretation was given to low ambulation scores in the open-field.

The response latency scores, however, did not predict the number of avoidances on the first day of training. Both HN and DNoN which had short latency responses had a low number of avoidances. Their number of avoidances resembled those of controls. All three groups averaged one avoidance in 15 trials. On the other hand, DN which had a long response latency had a high number of avoidances, Their avoidance scores were similar to HLN and HNoN. Though there were differences between groups on the number of avoidances on the first day, these differences were not reflected in any differences in the number of trials to reach acquisition criteria.

The lack of a relationship between response latency prior to the first shock presentation and the number of avoidances on the first day may reflect specific effects of shock. Shock typically elicits a freezing response in the rat (Anisman, 1975; Blanchard & Blanchard, 1969). There are two processes involved in avoidance behavior: associative and non-associative processes (Anisman, 1975). The latter processes involve behavioral and physiological processes, e.g. freezing. A limiting factor in acquisition is the ability of the rat to inhibit freezing tendencies (Anisman & Waller, 1973). Since groups with long response latencies during CS pre-exposure were characterized by freezing, these same groups should also show a low number of avoidances. The amount of activity exhibited following shock offset, however, is not predictable from activity prior to the first shock presentation (Anisman & Waller, 1972). Furthermore the amount of activity following shock presentations correlates with later avoidance performance. The dissociation of activity prior to shock and performance is exemplified in the present study. Though there were no observations of activity following shock, the differences in the number of avoidances on the first day may reflect differences in activity following shock: those groups with a low number of avoidances freezing more than those groups with a high number of avoidances.

Differences in activity following shock seems the best available explanation of the first day effect in the number of avoidances, particularly since there were no differences in the number of trials to acquisition. Anisman (1975) used a two day training procedure and attributed improvements on the second day to suppression of freezing tendencies. If there were differences in associative processes, this improvement would not be expected.

The observed differences in the number of avoidances on the first day are not clearly related to the experimental treatments. If the tendency to freeze is the limiting factor, it would appear from the data that prenatal stress either decreased or increased this tendency. The controls had a low number of avoidances as did two of the stress treatments, HN and DNoN. The similarity in these two treatments with the controls may reflect an increase in the tendency to freeze though an examination of this may be hindered by a floor effect. In the other prenatal treatments, DN and HLN, stress decreased the tendency to freeze as reflected in the increase in the number of avoidances.

Behavioral measures are necessary before these differences can be attributed to differences in freezing behavior. It is also recognised that the effect was transient so its importance is questionable.

Though there were no treatment effects in the number of trials to acquisition, there was a treatment effect on extinction. After 200 trials, the four animals in the HN group had not reached the extinction criterion compared to four of five controls which had. The inclusion of extinction in the current study was based upon evidence in the literature dealing with the effects of the pituitary-adrenal axis on avoidance behavior. There are studies which show a positive correlation between pituitary-adrenal function and resistance to extinction (DiGiusto et al., 1970; Johnston et al., 1974). Furthermore, the enhanced resistance to extinction in this literature has been interpreted as indicative of greater fear (Weiss et al., 1970). In the present study, however, the group HN which showed enhanced resistance to extinction was also the group which had the highest ambulation score in the open-field. The open-field data were interpreted as reflecting a decreased emotionaltiy. There appears to be a contradiction in interpretation: a less emotional rat is more fearful during extinction?

Within the literature of avoidance behavior there is evidence of a relationship between open-field behavior and avoidance performance: less emotional rats performing better than more emotional rats (Anisman & Waller, 1971;

Gupta & Holland, 1969; Reynierse, 1970). There are other studies which have not found any relationship (Barrett & Oakley, 1970; Joffe et al. 1972). One of the problems in establishing a relationship between emotionality and avoidance performance appears to be the use of strains in which high activity and low defecation measures are a characteristic of the strain (Gray & Lalljee, 1974). However, if there is a relationship between emotionality and avoidance performance, then the rapid extinction of more emotional animals may reflect greater fearfulness. Extinction can occur even though fear as measured in a conditioned suppression paradigm is high (Kamin, Brimer, & Black, 1963). Fear may even precipitate extinction if it results in a freezing response (Denny, 1971, pp. 256-257; Kimble, 1961, pp. 308-309). However, if fear can precipitate extinction, what variables maintain avoidance behavior during extinction?

Both the relationship between emotionality and avoidance, and the fact that extinction occurs at moderately high levels of fear contradict the assumption that high levels of corticosterone and enhanced resistance to extinction reflect greater levels of fear. However, it is not clear from the pituitary-adrenal literature whether the differences in extinction reflect differences in fear (emotionality) or memory processes (de Weid, 1974).

In the present study, the enhanced resistance to extinction of the HN group could be interpreted as reflecting lowered emotionality. This supports the interpretation of the open-field activity. However, such an interpretation ignores the fact that avoidance behavior involves both associative and non-associative processes. Except for the fact that prenatal stress effects are generally interpreted in terms of changes in emotionality (Archer & Blackman, 1971), there is no logical reason to exclude alterations in associative processes.

In conclusion, response latency to the CS alone, initial number of avoidance responses, and number of trials to extinction yielded significant results. The ordering of treatments in terms of latency scores to the CS alone was very similar to the orderings of treatments in terms of ambulation scores in the open-field. It was concluded that both measures reflected differences in reactivity. However, in contrast to the noise treatment effect in ambulation, the short latency scores were due to the experimental procedure rather than to noise stimulation. The effect in the initial number of avoidances occurred only on the first day. The effect was attributed to the presence of shock causing subjects in some treatments to freeze during the CS. There was no difference, however, in the number of trials to reach acquisition criteria. The only effect on extinction occurred in the HN group which showed an increased resistance to extinction. The effect was dependent upon noise stimulation of a hearing mother.

# GENERAL DISCUSSION

This thesis was addressed to the problem of whether sounds have predominantly direct or indirect effects on the rat fetus. The complexity of the mammalian maternal/fetal environment makes it impossible to exclude an interaction of indirect and direct effects. The question was whether effects were biased more in one direction than the other.

There were six treatment groups in the design: HN, HNoN, DN, DNoN, HLN, and controls. The control group was the baseline against which the experimental conditions were compared. HNoN and DNoN were additional controls for the noise stimulation groups, HN and DN respectively. The HLN group was another control for the DN group. The HLN group was included due to the uncertainty of the amount of hearing loss due to the experimental deafening procedure. Consideration would have to be given to direct effects if the behavioral measures of the DN offspring were significantly different from the DNoN group. If, however, the behavior of the DN offspring was not significantly different from the HLN offspring, this finding would mitigate somewhat against a direct effect. It would indicate that the deaf noise females probably perceived sounds. Thus their response to the noise could be mediating the effect.

The offspring were tested in three different behavioral tests: the open-field, shuttle box, and one-way avoidance. The shuttle box experiment yielded no differences between treatments.

Of the six behavioral measures taken in the open-field, only two resulted in any treatment effects: ambulation and grooming duration. There were two components to the treatment effect on ambulation: a noise stimulation effect and an experimental procedure effect. The noise stimulation effect occurred in the HN group. This was the only group to differ significantly from the controls. The effect depended upon the hearing ability of the mother and noise stimulation. DN, DNoN, and HLN did not differ from one another. The experimental procedure effect was present in all treatment groups. Any aspect of the experimental procedure increased the activity of the treatment groups above the controls. The increased activity in the offspring of the experimental treatment groups was interpreted as indicating a decrease in emotionality. The grooming duration was dependent solely upon the DN group. Whereas all other groups showed an increase in grooming across days, the DN group showed a steady decrease across days. This effect was difficult to interpret.

The avoidance procedure was used as a further test for examining prenatal stress effects. Previous studies (Joffe, 1969; Joffe et al., 1972; Smith et al., 1975) have shown prenatal stress effects in the number of avoidances. In this study, there were no differences in the number of trials to acquisition. However, the particular procedure used yielded some interesting findings which would have been overlooked if attention had been confined solely to the number of trials to acquisition.

Three measures on the one-way avoidance task yielded significant effects: latency of response to the CS alone, number of avoidances on the first day, and number of trials to extinction. The latency of response to the CS alone was interpreted as a measure of reactivity. This interpretation was based on the casual observations of the rats' activity during CS onset/offset and the similarity in the response latency scores to the activity scores in the open-field. Those groups with short latencies had high activity scores. Since none of the experimental groups differed from each other, it was concluded that it was the experimental procedure and not noise stimulation per se which resulted in decreased latencies.

The effect on the first day in the number of avoidances was due to two distinct groupings: those rats with a high number of avoidances and those rats with a low number of avoidances. It was proposed that the differences in performance between the treatments was due to differences in reaction to shock. Those groups with a low number of avoidances tended to freeze. Prenatal stress relative to the controls increased the number of avoidances in some groups. However, it also decreased avoidances in other groups. There was no apparent pattern between the experimental treatments and the number of avoidances.

Though there was no apparent relationship between the experimental treatment and the number of avoidances on the first day, the prenatal treatment effect was clear on extinction. The condition for enhancing resistance to extinction depended upon noise stimulation of a hearing mother. Whether the effect on extinction reflected differences in pituitary-adrenal function, however, will require measurement of corticosterone levels. Enhanced resistance to extinction has been associated with high levels of corticosterone and has been interpreted as indicating a greater level of fearfulness (Weiss et al., 1970). However, since the open-field behavior did not support a fearfulness interpretation, it was not clear whether the avoidance behavior reflected corticosterone differences. Despite the problems in interpreting extinction effects, it was proposed that the difference might reflect a difference in associative or memory processes.

The results from the avoidance task indicated that the use of a neutral CS as the maternal stressor did alter performance on tasks other than the open-field. Furthermore, the behaviors on the avoidance task supported the findings of the open-field: that noise stimulation of a hearing mother is an important component in this prenatal stress design.

These results taken together show that noise stimulation during gestation does have an effect on the later behavior of the offspring. The effect is mediated mainly through the mother. That noise stimulation was mainly indirectly mediated does not mean that there were no direct effects. There was the unexplained result on grooming. The DN group differed from all other groups. A conservative conclusion is that noise stimulation is an interaction of direct and indirect effects with the main effect resulting from the maternal reaction to noise stimulation. For a more conclusive statement, the deafness of the mothers would have to be evaluated as well as monitoring fetal activity during periods of stimulation.

This thesis also had bearing on another problem in the literature. Other studies have investigated the effects of neutral stimuli as maternal stressors (Jolley & Adam, 1975; Jolley & Dreesman, 1973; Morra, 1965b). Morra used a sound stimulus and found that the offspring were more emotional. Jolley and his co-workers used a light and found that the offspring were less emotional. The different effects of these two stimuli on the offsprings' emotionality raised the question as to whether there were differences in mediation: sound being directly mediated, and light being indirectly mediated. The present study, however, demonstrated that sound is indirectly mediated. Hence differences in mediation cannot account for the emotionality differences. Instead, it was proposed that differences in the amount of disturbance or stress that the mother undergoes is the critical factor. Using the present procedure of sound presentation, future studies could examine whether there are differences when presentations are given in a completely novel environment, in the home cage in a novel chamber, and in the holding room. If there are differences, then perhaps prenatal stress effects can be conceived of as reflecting differences in amount of maternal stress just as early experience effects are thought to reflect varying degrees of stimulus input (Henderson, 1969).

The objection that sound might have direct effects on the fetus and thus be a confounding variable in conditioned avoidance procedures using a buzzer is not supported by this thesis. Rather, the prenatal stress effects found in these studies reflects a maternal psychological stress response. This is not to say that premating stress is not a factor in the conditioned avoidance effect. The problem still remains as to what physiological processes of the mother are affecting the fetus. The use of a procedure which results in little disturbance of the maternal environment may facilitate research in this area.

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