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in the RatUNIVERSITY/UNIVERSITÉ Carleton UniversityDEGREE FOR WHICH THESIS WAS PRESENTED/
GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE Doctor of PhilosophyYEAR THIS DEGREE CONFERRED/ANNÉE D'OBTENTION DE CE DEGRÉ 1975NAME OF SUPERVISOR/NOM DU DIRECTEUR DE THÈSE Bruce A. Pappas

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Prenatal Stress:
Effects on Somatic
and Behavioral Ontogeny
in the Rat

by

Sonya Krishna Sobrian

A thesis submitted to the Faculty of
Graduate Studies in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Department of Psychology

Carleton University

Ottawa, Ontario

The undersigned recommend to the Faculty
of Graduate Studies acceptance of the thesis

"Prenatal Stress:

Effects on Somatic
and Behavioral Ontogeny

in the Rat"

submitted by Sonya Krishna Sobrian
in partial fulfillment of the requirements for
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Prenatal Stress:
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and Behavioral Ontogeny
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Sonya Krishna Sobrian

An Abstract of a Thesis presented to the
Faculty of Graduate Studies of Carleton
University in partial fulfillment of the
requirements for the degree of
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Ottawa, Ontario

The purpose of this thesis was to determine whether physical stress applied to the pregnant female rat would alter behavioral and/or biochemical ontogeny of the offspring.

Previous research has indicated that a variety of prenatal stressors can alter the adult behavior of the offspring. Prenatal maternal stress (PMS) has generally been reported to increase offspring emotionality, as indicated primarily by increased open field ambulation and defecation scores. However, the opposite results have also been reported. While there is some evidence that the performance of PMS offspring on several learning tasks is better than that of controls, the majority of studies have reported that PMS offspring are inferior to controls.

The first two experiments reported here examined whether PMS might also alter early behavioral development and biochemical development of the CNS, as reflected by biogenic amine levels. The third experiment was designed primarily to verify the relationship between the behavioral variables used in Exps. I and II and CNS development.

Exp. I Female rats were mated and their pregnancy status was determined by vaginal smears. During days 8-14 of gestation, the pregnant females were subjected to either 30 minutes of handling or to 15 unsignalled electric foot shocks presented on a 2-minute variable interval schedule. Some of the females were subjected to vaginal smears but were not disturbed during gestation. Within 48 hours of birth, all of the litters from these mothers were cross-fostered to non-sméared, non-stressed primiparous females.

Using a between subjects design, offspring were tested every second day up to 21 days of age for the appearance of incisor eruption and eyelid dysjunction, the maturation of the free-fall righting reflex and the startle response, and the development of spontaneous locomotor activity (SMA). The latter behavior was chosen because it has been shown to exhibit a characteristic ontogenetic pattern. At 30 and 60 days of age, open field behavior was examined.

Somatic and reflex development were not altered in PMS offspring. However, overall activity levels during the neonatal period were lower in the offspring of PMS mothers in comparison to controls. It was difficult to make a definitive statement concerning the effects of PMS on the ontogeny of locomotor arousal in that the age-related changes in activity seen in this experiment might have reflected locomotor activity that was elicited by the novelty of the testing situation.

Open field ambulation was decreased in the offspring of mothers subjected to only vaginal smears and to vaginal smears plus handling. This generally supported prior reports. However, the offspring of mothers subjected to electric shock showed increased open field ambulation and this was attributed to the high degree of stress to which females in this condition were exposed due to the inescapable and unpredictable nature of this stressor. In addition, PMS altered the adaptation rates of ambulation, rearing and grooming behavior in the open field.

Exp. II This experiment was designed to determine whether cross-fostering, the trimester during which stress was administered, or the sex of the pup would modulate the effects of PMS. It also

investigated the effects of PMS on the maturation of spontaneous alternation (SA) behavior and on the development of endogenous levels of norepinephrine (NE), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA). These amines have been correlated with the maturation of both SMA and SA.

Female rats were mated and removed from the males after 5 days. Vaginal smears were not used to determine pregnancy status as the results of Exp. I suggested that this also affected offspring behavior. Pregnant females were exposed to electric foot shock either from days 6-12 or 15-21 postmating. At birth, half of the litters were fostered to non-stressed control females and the remainder were left with their biological mothers.

From a repeated measures design, which was used to attenuate the response of the pups to the novelty of the test situation, the ontogeny of SMA appeared to be accelerated in PMS offspring. Cross-fostering of stressed pups accentuated the difference between PMS and controls. Prenatal stress applied during the second trimester was more effective in accelerating the development of SMA but only in the cross-fostered pups.

The maturation of SA was delayed in both fostered and non-fostered PMS pups. As in Exp. I, somatic and reflexive development was not altered. An explanation of these results was formulated in terms of a possible precocial development of the CNS with a subsequent deficit in brain weight and total cell number in prenatally stressed rats.

Alterations in the development of endogenous concentrations

of NE, 5-HT, and 5-HIAA were not evident. Control and PMS groups exhibited the characteristic increase in steady-state levels as a function of age. The sex of the offspring did not interact with any of the variables.

Exp. III This experiment was primarily designed as a methodological study to determine whether the behavioral variables used in the previous experiments were sensitive to alterations in CNS development. The biochemical and anatomical alterations seen after neonatal thyroidectomy make the hypothyroid rat an ideal preparation in which to assess the relationship between behavioral ontogeny and CNS maturation.

Hypothyroidism was induced in the neonatal rat by a single i.p. injection of 200 micro-curries of radioactive iodine at birth. Controls received an injection of saline.

The ontogeny of both SMA and SA as well as somatic and reflexive development was retarded in the cretinous pups. These findings were consistent with the reports of delayed brain maturation. In addition to confirming the relationship between behavioral and biochemical development, the results of Exp. III provided the first experimental evidence for the putative role of the thyroid gland in behavioral ontogeny above the reflex level.

Acknowledgements

I would like to express my sincere appreciation to Dr. Bruce A. Pappas for his support, guidance and intellectual stimulation throughout the preparation of this thesis. I also wish to thank him for the use of his laboratory facilities and personnel.

My gratitude is extended to Dr. William G. Webster whose encouragement and comments on this thesis were invaluable.

Experiment III would not have been possible without the co-operation of the members of the Department of Pharmacology, University of Ottawa. In particular, I would like to thank Dr. Radhey L. Singhal for the use of his laboratory facilities and for providing the radioactive iodine and the animals used in that study, Dr. David A.V. Peters for his biochemical expertise and the use of his laboratory and supplies during the exploratory phases of this thesis, Dr. Irene Mazurkiewicz-Kwilecki for the use of her equipment and Dr. Ram B. Rastogi for his assistance with the injection procedure.

I am deeply indebted to Ms. Barbara Drew for her biochemical and technical assistance in the preparation of this thesis.

Thank you to Christine McClymont and Maureen Quinn for the use of their apartment facilities and equipment during the final stages of this thesis.

This research was supported by a Pre-doctoral Research Studentship from the Ontario Mental Health Foundation.

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INTRODUCTION

That the developing organism is capable of undergoing morphological, biochemical and behavioral changes postnatally is well established. Historically, the question of whether these alterations represent a genetically determined delayed maturational process, or whether they are influenced by a pattern of interaction between the developing organism and its environment has been formulated in terms of polar alternatives, i.e., nature versus nurture, instinct versus intelligence, and innate versus learned. Hinde (1970) has elaborated upon the sterility of such dichotomies and pointed out that the determination of developmental characteristics consists of a "nexus of causal relations with action, reaction, and interaction, both within the organism and between the organism and environment at every age" (p. 425). The factors involved in these complex interactions have been conveniently, if not expediently, grouped into hereditary and environmental categories and consequently research in this area has addressed itself to determining if observed differences between two organisms can be ascribed to genetic or experiential factors. However, Bekoff and Fox (1972) have suggested that the issue that should be considered is how maturational processes are influenced by interaction with the environment.

The environment was originally thought to play a passive role during development, supporting and nurturing an organism that was genetically pre-programmed to develop, mature and express itself in a pre-determined manner. However, recent evidence has illustrated that

the environment has dynamic properties which can interact with genetic potential to contribute towards specifying the ultimate characteristics and capabilities of a developing organism. An enriched environment during early postnatal life has led to improved problem solving and learning performance (Cooper and Zubek, 1958); prenatal and postnatal malnutrition can reduce the number of brain cells and retard the development of reflexive behavior (Smart and Dobbing, 1971); mildly stressful stimulation during the neonatal period reduces emotionality and produces better physiological adaptation to stress in the adult (Thompson and Grusec, 1970); the pregnant female's experiences during the prenatal period can influence both the behavior and physiology of the offspring (Joffe, 1969). However, the degree to which treatments can influence the maturation of the organism is set within the limit of the genotype. This genotype can be permissive or non-permissive to environmental influences in the sense that it may allow very small or very large deviations from a predicted developmental pathway.

Although the importance of early environmental influences has been acknowledged, the question of how these manipulations can alter adult behavior has received little attention experimentally. This thesis is addressed to that question. It was hypothesized that changes in adult behavior following early manipulations are the result of disruptions in the ontogeny of behavioral processes which reflect possible alterations in the postnatal development of the central nervous system (CNS). This was examined by subjecting pregnant female rats to one of several environmental stressors. Several indices of behavioral and

somatic development were used to determine the effects of this prenatal stress on the ontogeny of selected behaviors. Biochemical determination of endogenous amine levels were also carried out to explore the possibility of changes in CNS maturation.

Prenatal Influences and Behavior

The theory most often encountered in historical reviews of the prenatal determinants of behavior is that of maternal impressions, which is the idea that almost any event influencing the pregnant organism can affect the course of pregnancy and disposition of the unborn fetus. Rather than ascribing the outcome of pregnancy to magic or the whims of temperamental deities, it stresses the mediational role of the maternal organism. It was her behavior and her environmental experiences that were potentially capable of affecting the unborn organism.

The theory of maternal impressions is both ancient and ubiquitous. References to this notion are recorded in the Bible (Genesis:30) and appear in the oral and written tradition of Europe and the Americas as well as in India, China and Japan, and among Eskimos and Africans (Ferreira, 1969; Graham, 1951). However acceptance or rejection of the idea has fluctuated over the centuries and has depended upon the prevailing philosophical and religious climate rather than the findings of empirical inquiry.

Original interest in the effects of prenatal manipulations were influenced by embryological methods and emphasis was placed on changes in the physical development of the fetus that resulted from manipulation

of the female during gestation. While the incidence of physical abnormalities in the offspring that result from exposure of the pregnant animal to environmental factors such as disease (rubella) or drugs (Thalidomide) is well documented (Kalter, 1968), the effects of these agents on the behavior of the offspring has been largely ignored. It was not until 1963 when Werboff and Gottlieb introduced the concept of "behavioral teratology" in reference to the administration of psychoactive drugs to pregnant rats, that both the physiological and behavioral consequences of these agents were recognized.

Since then, studies aimed at establishing the behavioral effects produced by various prenatal treatments have proliferated. In addition to drugs and disease, interest has grown in elucidating the effects of agents such as noise, temperature variation, changes in oxygen supply, and X-irradiation during pregnancy on the behavior of the offspring. Furthermore, manipulation of the psychological environment in such a manner as to evoke an affective response in the pregnant organism, has been shown to influence subsequent behavior in the offspring.

The majority of studies concerned with the results of prenatal manipulations has concentrated on subsequent alterations in the adult behavior of the offspring. Research on developmental processes has been limited for the most part to changes in somatic and reflexive responses.

Several authors have provided recent reviews of the literature of a variety of prenatal treatments which have produced behavioral changes in the offspring in the absence of observable

morphological damage (Ferreira, 1969; Joffe, 1969; Thompson and Grusec, 1970; Archer and Blackman, 1971). These prenatal treatments are believed to produce changes in the behavior of the adult offspring by altering the internal milieu of the fetus as a consequence of non-specific hormonal changes produced by activation of the sympathetico-adreno-medullary and pituitary-adreno-cortical axis of the mother in response to stress. Since this thesis is concerned with the effects of prenatal physical or psychological stress, the following review will concentrate on studies involving these manipulations. However, it will also include experiments in which other prenatal influences have been shown to produce alterations in several ontogenetic variables.

Prenatal Stress and Behavior

Almost any kind of treatment imposed upon the pregnant female can affect the fetus. The fact that the general direction of these effects is remarkably similar despite the qualitatively different nature of these procedures suggests that any manipulation that can produce a stress response in the pregnant female is capable of leaving a residue on the developing organism that can influence subsequent behavior.

The classic experiment designed to examine the effects of prenatal maternal stress (PMS) was conducted by Thompson (1957). Female rats were trained first to expect shock at the sound of a buzzer in one side of a two compartment box, then to avoid shock by opening a door and running through to the other side. The assumption was that this procedure produced an acquired fear response in the female. After

reaching a certain criterion of responding, females were mated and throughout the gestation period were given three daily exposures to the buzzer. The avoidance response, which had previously led to trial termination, was blocked. The 'offsprings' behavior was tested at 30-40 and 130-140 days in an open field situation. Ambulation and defecation scores served as indices of emotionality. The low activity and increased defecation of experimental offspring suggested that fear elicited during pregnancy increased emotionality of the offspring.

Although these general results have been replicated by a number of investigators (Doyle and Yule, 1959; Hockman, 1961; Ader and Belfer, 1962; Thompson, Watson, and Charlesworth, 1962), decreases as well as no change in emotionality have also been reported (Thompson and Quinby, 1964; Corson, Heseltine, and Smith, 1970, respectively). The direction of the prenatally stressed offspring's emotional response is influenced by the strain of the animal. Reports of increased emotionality in Long-Evans rats (Hockman, 1961; Ader and Belfer, 1962) contrast with reports of reduced emotionality in Sprague-Dawley rats (Thompson et al., 1962; Hutchinson and Gibbon, 1970). In addition, fostering may interact with prenatal stress to either facilitate or inhibit the results of the latter (Thompson, 1957; Hockman, 1961; Ader and Belfer, 1962; Thompson and Quinby, 1964; Joffe, 1965). A number of prenatal parameters (i.e., duration and timing of stress) as well as postnatal environmental conditions (i.e., age at testing and prior test experience) have been shown to interact with prenatal stress in terms of offspring effects (Archer and Blackman, 1971).

Maze learning, operant behavior, and certain physiological responses have also been found to be altered by maternal conditioned fear. Thompson et al. (1962, 1963), Thompson and Quinby (1964), and Morra (1965) investigated the effects of prenatal conditioned fear on the performance of offspring in a water maze. In all three studies, trials to criterion learning were not affected by the prenatal treatment. However, Thompson and his colleagues (Thompson et al., 1962, Exp. 3; Thompson and Quinby, 1964) reported that PMS generally increased time scores and errors per trial. In later studies, PMS offspring learned a water maze with decreased time and errors per trial than controls (Morra, 1965). In addition, there are several reports that PMS did not alter time (Thompson, Goldenberg, Watson, and Watson, 1963) or error scores per trial (Thompson et al., 1963; Thompson and Quinby, 1964). These discrepant results reflect a difference in test conditions, age and prior experience of the offspring among the experiments.

The acquisition of a two-way conditioned avoidance habit is facilitated in the offspring of stressed mothers (Joffe, 1965). This finding would correspond with the reports of increased emotionality if a greater degree of fear in an avoidance situation facilitated avoidance learning. However two way shuttle avoidance performance is impaired in offspring of mothers exposed to the conditioned situation (Corson et al., 1970). Gastric ulceration after immobilization stress and food deprivation was increased in rats whose mothers were subjected to conditioned avoidance at the time the fetal gut was forming (Bell, Henry,

and Miller, 1967).

Various types of maternal physical stressors seem to produce qualitatively similar offspring effects. Increases in emotionality, as measured by either decreased open field activity or increased response time on a cage emergence test have been reported in the offspring. These changes have been reported following the crowding of pregnant mice (Keeley, 1962), and the administration of swim, tilt and open field stress to pregnant rats (DeFries, 1964; DeFries and Weir, 1964) and the exposure of pregnant rats to extremely low frequency electromagnetic fields (Persinger and Pears, 1972; Ossenkopp, 1972; Ossenkopp, Koltek, and Persinger, 1972). However, handling pregnant rats (Ader and Conklin, 1963) decreased the emotionality of their offspring as evidenced by their decreased response to handling, and their decreased defecation in the open field (Chiszar, 1971). Changes in plasma corticosterone after handling reflected this decreased reactivity in 90 day old offspring. While open field activity was not altered in the offspring of females exposed to either a conflict stress (Joffe, 1965) or subjected to audiogenic seizures (Thompson and Sontag, 1956) during gestation, these two stressors disrupted the maze learning performance of the pups.

Two studies have investigated developmental changes following prenatal manipulation. The age at which eyelid dysjunction and incisor eruption occurred was delayed in the offspring of pregnant females exposed to low frequency electromagnetic fields (Ossenkopp et al., 1972). The handling of pregnant rats accelerated the characteristic 24-hour rhythm in adreno-cortical activity. Behavioral maturation was also

advanced. The 24-hour rhythm in overt day-night activity, characteristic of the adult rat, was first observed at 7 days of age in control pups, whereas prenatally handled animals displayed rhythmicity at 5 days of age (Ader and Deitchman, 1970).

All of the above studies have concentrated on the effects of prenatal stress on adult behavior. With two exceptions, the immediate consequences of PMS in animals have been ignored. Theoretical formulation of the dependence of adult behavior, primarily learning, on the experience of the young organism has been made by Hebb (1949), Brookshire, Littman and Stewart (1961) and more recently Thompson and Grusec (1970). Empirically, behavioral patterns seen in the adult have been shown to have their antecedents during embryonic development (Hamburger, 1968). Specific movements or reflexes appear first and these form the elements out of which later complex behaviors are constituted (Sullivan, 1967; Fox, 1965).

Since PMS is capable of affecting adult behaviors and these adult behaviors appear to have their antecedents during embryonic and early postnatal development, I hypothesized that the observed changes in adult behavior, as a function of PMS, are the consequence of altered patterns of maturation during the neonatal period. This thesis was therefore designed to examine the effects of PMS on postnatal behavioral development. The suggestion is that the initial description of the ontogenic process occurred in utero.

The Ontogeny of Behavior

Despite the voluminous experimental literature on the rat, there

were until recently very few data concerned with a basic description of behavioral development in the neonatal animal. The belief that the behavioral repertoire of the altricial rodent is too limited to warrant any study before 15 days of age was probably responsible for this lack of information. However, in the 5-15 day postnatal rat, neuro-anatomical, neurophysiological and biochemical changes are occurring at a pace that is perhaps more rapid than at any other time during development (Himwich, 1962). Yet behavioral research on the pre-weanling rat is sparse. Most research concerned with behavioral changes as a function of age has involved animals of weaning age or older - ages at which a major portion of brain maturation has already occurred. While providing interesting age differences in behavior, it has not led to any definitive information on the emergence of various response capabilities.

Much of the data now available on the ontogeny of behavior come from the work of Campbell and his associates (Campbell and Riccio, 1966; Campbell, 1967; Moorcroft, Lytle, and Campbell, 1971; Campbell and Spear, 1972; Campbell and Mabry, 1972, 1973; Mabry and Campbell, 1973). They have systematically studied changes in various behaviors as a function of age, beginning at birth, in several species, and have formulated an ontogenetic theory of behavioral arousal in the rat incorporating neurochemical ontogeny.

Campbell's Theory of Neurochemical-Behavioral Ontogeny During the ontogeny of behavioral arousal, the neonatal rat exhibits a period of intense excitability. Spontaneous locomotor activity (SMA) increases

dramatically during the first two weeks of life, reaching maximal levels at 15 days of age. It then declines to adult levels at 25-30 days of age (Campbell, Lytle, and Fibiger, 1969; Campbell and Mabry, 1972). This has been confirmed in our laboratory (Sobrian, Weltman, and Pappas, 1975). Campbell et al. have correlated these changes in activity levels with structural and functional development of the CNS. Based on work with d-amphetamine, which enhances noradrenergic transmission, and scopolamine, which blocks acetylcholine (Ach) receptors, Campbell and his associates have hypothesized that the age-related changes in activity in non-drugged animals reflect, in order of appearance, the maturation of noradrenergic brainstem excitatory centers and the maturation of Ach-mediated telencephalic inhibitory centers. Further experiments with para-chlorophenylalanine, an inhibitor of serotonin (5-HT) synthesis, suggested the existence of a limbic-based serotonergic inhibitory system, possibly in the hippocampus (Trimbach, 1972), whose maturation precedes that of forebrain structures. They concluded that the sharp increase in activity seen at 15 days of age was correlated with the maturation of noradrenergic arousal systems and the transient weakness in normal inhibitory processes. The decline in activity seen after this age reflected the modulating effects of late-maturing inhibitory structures.

The implication of this theory of neurochemical-behavioral ontogeny is that the emergence of integrated behavior is dependent upon the development of several neurochemical systems and the cytoarchitectonic maturation of various structures in the CNS. Campbell and Mabry (1972,

1973) admit that increased SMA could reflect any one or a combination of several different processes other than the changes in CNS excitation and inhibition. These include the maturation of skeletal musculature, glandular development and an increase in sensory input. Moreover, Bronstein, Neiman, Wolkoff, and Levine (1974) have recently proposed an ontogenetic theory of behavioral inhibition based on experiential rather than physiological or biochemical variables. Changes in activity levels are thought to reflect primarily maternal-litter and intra-litter interaction. They proposed that the increased locomotor activity seen in the 15 day old rat reflected the interaction of "the ontogeny of ambulation and of independent eating..." (p. 96).

Spontaneous Alternation and Maturation of Limbic-Based Inhibition Spontaneous alternation (SA) consists of the tendency of an adult animal on two consecutive unrewarded trials to enter opposite arms of a T-maze (Dember and Fowler, 1958). This behavior also exhibits a distinct ontogenetic pattern (Douglas, Peterson, and Douglas, 1973; Egger, 1973a,b; Egger, Levesey, and Dawson, 1973). At 15-17 days of age, animals continually choose one goal arm over the other (perseverate behavior). Chance levels of alternation are seen at approximately 22 days of age. Reliable levels of alternation behavior first appear between 28 and 30 days postpartum. The alternation of selected goal arms increases from approximately 20% in 15-17 day old rats to 80% in the adult.

Spontaneous alternation has been linked with hippocampal-mediated response inhibition. The ability to alternate response choices is disrupted by lesions of the hippocampus in the adult rat (Stevens,

1973). This deficit is believed to reflect the inability of the animal to inhibit prepotent responses (Eggar et al., 1973), or the loss of a response braking mechanism (Altman, Brunner, and Bayer, 1973). The ontogeny of SA seems to involve the structural and functional development of the hippocampus. Interference with the postnatal acquisition of the granule cells of the hippocampus by X-irradiation significantly disrupted the acquisition of alternation behavior in the rat (Bayer, Brunner, Hine, and Altman, 1973). In the precocial guinea-pig, which is born with a large population of differentiated hippocampal granule cells, SA reaches adult levels at 10 days of age. In the rat, hippocampal neurogenesis in the dentate gyrus is evident until approximately the end of the 4th postpartum week, an age at which the majority of rats begin to show adult levels of alternation behavior (Douglas et al., 1973). Thus, the perservative behavior of younger rats is thought to reflect as yet undeveloped inhibitory mechanisms which are mediated by microneurons of the hippocampus (Altman et al., 1973).

The ontogeny of SMA and SA were selected as dependent variables in this thesis because they provide useful developmental norms for behavioral variables against which the effects of prenatal treatments can be compared. They are also, at least theoretically, unlearned responses. Therefore any age related changes in these behaviors should reflect maturational rather than experiential factors. Motivational factors should, likewise, minimally affect them. Finally, the maturation of these behaviors in the neonate can serve as a behavioral index of the development of the CNS.

In addition to using these two variables as general indices of behavioral development, the age at which eyelid dysjunction and incisor eruption occurred served as indices of somatic development. The startle response and the free-fall righting reflex were used to assess the maturation of reflexive behavior (Eayrs and Lishman, 1955; Smart and Dobbing, 1971).

The Plasticity of Behavioral Ontogeny

That plasticity of the developing nervous system may occur in response to the interplay between genetically determined and environmental factors during embryological and early postnatal development is well documented for the visual system (Hubel and Wiesel, 1965; Holloway, 1966; Cragg, 1969; Fifkova, 1970; Hirsch and Spinelli, 1970). Moreover, increases in the number of dendritic spines in the rat's cortical pyramidal cells have been reported following early stimulation (Schapiro and Vukovich, 1970). Infantile handling has been shown to increase cell proliferation and the formation of new microneurons in the cerebral and cerebellar cortices of the rat (Altman, Das, and Anderson, 1968).

Several lines of evidence indicate that developing behaviors are also sensitive to alteration by postnatal and prenatal manipulations. Infantile stimulation can influence the maturation of the stress response as indexed by adrenal ascorbic acid depletion (Levine and Lewis, 1959) and plasma and adrenal corticosterone (Levine, 1962). Handled subjects showed a decrease in adrenal ascorbic acid concentrations as early as 12 days of age, while cold stress did not induce significant depletion in undisturbed animals until the 16th day. Shock-induced increases in

plasma corticosterone appeared in stimulated subjects by the third day of life. In contrast, non-handled animals were a week old before the response was seen. These findings indicate that neonatal manipulations can accelerate the maturation of the adrenal response to stress. This accelerated development might be the mechanism which mediates improved performance in an emotion-provoking situation in the adult animal.

The rate at which an organism habituates to a novel stimulus increases monotonically with age (Douglas et al., 1973; Bronstein et al., 1974). Snedden (1971) tested the hypothesis that supra-normal stimulation could accelerate the normal developmental sequence of the habituation process. Exposing animals to auditory stimulation for either one or five weeks prior to testing at 25, ~~55~~, and 85 days of age, increased the habituation rate of experimental animals above controls at all ages and also increased the habituation rate of younger animals to that seen in the adult animal.

The maturation of the rat's cardiac response to auditory stimulation involves a decremting in heart rate at stimulus onset; with repeated presentation of a tone stimulus, the response habituates (Ashida, 1973). Midgley (1974) found that infantile experience accelerated the age at which a reliable decrease in heart rate was observed in response to an auditory stimulus. This decremting response was first observed as early as 10 days of age in handled animals. In controls, this response was not statistically reliable until day 20.

Early nutritional deprivation adversely affects the ontogeny of a large number of reflexes and the course of development of explor-

atory responses (Smart and Dobbing, 1971). Maternal food restriction during the last week of pregnancy and throughout lactation significantly delayed the appearance of eye-opening and incisor eruption and the development of the palmar grasp, the auditory startle response, free-fall righting and visual placing. Undernourished rats were also significantly older than controls before they were seen to display each of six exploratory responses in the open field.

Research involving the effects of prenatal treatment on behavioral ontogeny has been conducted primarily with birds. For the mallard, the onset of the embryo's discriminative response to their own species' call occurs on days 22-25 (Gottlieb, 1970). Gottlieb (1970) selectively altered the normally occurring perinatal auditory experience, and found that the normal course of events could be changed. Embryos placed in auditory isolation on day 23 did not show the usual increase in bill clapping or vocalization rate when presented with the mallard call on days 24 and 26. Embryos prenatally exposed to sibling vocalizations on days 21 and 22 showed a reliable premature tendency to increase the rate of bill clapping in response to the mallard call on day 23.

Prenatal administration of drugs has also been shown to alter the behavioral response of developing organisms (Sparber and Shideman 1970; Sparber, 1972). Small doses of reserpine injected into fertilized eggs before incubation prolonged the time required for hatching. Both vehicle and non-injected eggs exhibited a distribution of hatching time that was skewed to the left, with approximately 70% of the chickens hatching during the first half of the total hatch period. Injection of

0.4 or 0.5 mg of reserpine reduced the percent of eggs hatching during the first half of the period to 9%. The characteristic imprinting response seen in chickens was also delayed. In the reserpine treated chickens, the age at which maximum following occurred was 25 hours after hatch as compared with the 16 hours normally observed. When control and treated chickens were tested on two-way shuttle avoidance at 7 days of age, initial rates of avoidance responding did not differ. However, within three days, the controls were performing better than the treated birds.

In mammals, drugs administered to the female during gestation can also influence behavioral ontogeny. The offspring of female rats injected with increasing doses of morphine prior to conception and for two weeks of gestation displayed increased activity levels over control pups and an extended period of hyperarousal, with activity increasing sharply between 10 and 15 days of age and remaining elevated until day 25. The initial decrease in controls' activity was seen at 20 days of age (Sobrian, Edson, and Pappas, unpublished observations).

The above experiments indicate that the progression through certain ontogenetic stages of development can be influenced by environmental factors applied to the developing organism. This effect can be seen in precocial and altricial species, in placental animals and in birds. It can be produced by prenatal and neonatal stimulation to influence physiological and behavioral development. There is also some suggestion that these alterations in the normal development of the immature organism can mediate changes in adult behavior.

The experiments therefore focused on the problem of whether pre-natal physical stress would alter the ontogeny of locomotor arousal and spontaneous alternation. If so, would these changes be reflected in adult open field behavior? The development of reflexes, physical features, and body weight were also evaluated to determine the extent to which somatic systems were affected. Changes in CNS maturation were assessed by determining endogenous levels of several brain amines during the neonatal period.

Experiment 1

In this experiment, pregnant rats were handled or exposed to electric foot shock for 30 minutes daily during the second trimester. Both stressors had previously been shown to produce changes in offsprings' behavior when administered to the pregnant female (Joffe, 1969). The period between days 8-14 of gestation was chosen as the interval for treatment because it is considered the most critical for brain organogenesis in the rat (Petropoulos, Vernadakis, and Timiras, 1968). Pregnancy was determined by vaginal smears. At birth, stressed litters were fostered to non-stressed control mothers.

The development of locomotor activity served as the primary behavioral variable used to assess alterations in ontogeny in the PMS offspring during the neonatal period. Emphasis was placed on this dependent variable because the developmental pattern reported by Campbell et al. (1969, 1972, 1973) had been replicated in our laboratory (Pappas, Peters, Sobrian, Blouin, and Drew, 1975; Sobrian, Weltman, and Pappas, 1975). Somatic and reflex development were assessed using the method of Smart

and Dobbing (1971). The appearance of eyelid dysjunction and incisor eruption, and the development of free-fall righting and the startle response have been shown to be sensitive to prenatal manipulation (Smart and Dobbing, 1971; Ossenkopp et al., 1973).

Open field behavior was evaluated in 30 and 60 day old offspring in order to determine if there was a relation between possible changes in neonatal activity and alterations in adult behavior. In addition to recording overall activity, rearing, grooming and boli counts, which allowed for a partial replication of existing PMS studies, adaptation rates of these variables were calculated. Archer (1973) has suggested that the use of adaptation rates provides for the assessment of changes in behavior over successive exposure to the open field without reference to constructs of emotionality or exploration.

PROCEDURE

Subjects

Adult (225-250 gram) nulliparous female Wistar rats (Rattus norvegicus) were mated to Wistar males in the ratio of 3:2, 48 hours after delivery to the laboratory from Bio-Breeding Labs, Ottawa, Canada. Vaginal smears were collected every second day until females were determined pregnant. This was designated day 1 of gestation. At this time, females were randomly assigned to one of three treatment groups and individually housed in polyethylene cages (44 x 25 x 30 cm) with ad libitum water and Purina ~~rat~~ chow. All animals were maintained on a 12 hour reversed light-dark cycle (lights off at 0800 hours).

Females designated as control and foster mothers were not subjected to vaginal smears. They were removed to nesting cages 10 days after being placed with the males.

Stress Procedures

Two different stressors, handling and electric foot shock, were employed on days 8-14 of gestation.

Group I Non-Handled (N-H) Females in this group were subjected to vaginal smears post-mating and were not further disturbed throughout gestation.

Group II Handled (H) These animals were handled for 30 minutes per day on days 8-14 of gestation. Handling consisted of three 10 minute sessions in which animals were removed from their home cages for 30 seconds, and then returned for 30 seconds. The inter-session interval was

50-60 minutes.

Group III Electric Shock (ES) Fifteen unsignalled, inescapable electric foot shocks (0.5 mA for 0.5 seconds) were administered to animals in this group during a 30 minute test session. The shock source was a constant current neon grid scrambler unit manufactured by the Carleton University Science Workshop. Shocks were programmed (BRS Foringer) on a variable interval 2-minute schedule. The shock apparatus was a 63 x 27 x 26 cm plexiglas compartment with a stainless steel grid floor. It was housed in a sound attenuated chamber and was cleaned and sprayed with Lysol between each rat to eliminate odor trails. A fan, mounted on the outside of the chamber provided ventilation and a mild masking noise.

Fostering Procedure

The fostering of prenatal maternal stress (PMS) pups was necessary in order to separate the prenataally transmitted influences of maternal treatment from those which might be mediated postnatally. The use of a non-handled group, two qualitatively different levels of stress, two control conditions and a between subject design necessitated against using traditional fostering/cross-fostering procedures. This method involves three rearing conditions: a litter can be raised by its biological mother, by a mother from the same treatment group as its biological mother (fostering), or by a mother from a treatment group other than its natural mother (cross-fostering). Using this procedure, a minimum of 450 litters of at least 8 pups each would have been required.

Instead, all litters were fostered to mothers of a single type, (in this instance, non-smearred, non-treated primiparous Wistar females),

thus holding the postnatal environment and fostering experience constant for all litters.

Litters were inspected for births at 1000, 1400 and 2000 hours. The day a litter was found was designated as day 1. Pups assigned to testing on day 1 were tested within four hours after being found. They had been fostered immediately and allowed to nurse the foster mother at least once.

The remainder of the litters were fostered within 48 hours after birth. Approximately 75% of the litters were fostered within 12 hours of birth. If foster mothers were not available, PMS litters were discarded. All litters were culled to 8-10 pups.

This fostering/cross-fostering procedure required the addition of two groups to the experiment.

Group IV Control-Fostered (C-F) Females in this group had not been smeared or disturbed during pregnancy. At birth their pups were fostered to a similarly treated mother.

Group V Control Non-Fostered (C-NF) In order to assess the effects of the fostering procedure on the dependent variables, a fifth group was needed. The offspring of this group differed from those in Group IV only in that they were raised by their natural mothers.

The experiment was conducted in 12 replications of 18 pregnant females each. The rats were randomly assigned to one of the above conditions with three rats each in the N-H, H, and ES conditions. The remaining nine rats served as either control or foster mothers.

Offspring Testing

Infant Activity Male and female pups from each litter were tested every second day from birth to 21 days of age, and on days 25 and 30. Activity was recorded with a field capacitance movement sensing device (Griffiths, Chapman, and Campbell, 1967), which was sufficiently sensitive to record large torsional body movements. The rats were placed in a cardboard box containing wood shaving which rested on the sensing coil. The dimensions of the container varied with age of the animal: Days 1,3, and 5--7.5 x 7.5 x 5cm; Days 7,9, and 11--7.5 x 7.5 x 9.0cm; Days 13 and 15--8.7 x 10.0 x 15cm; Days 17,19,21,25 and 30--10.0 x 10.0 x 21.0cm.

A thin layer of fresh sawdust was placed in the bottom of the box before testing. The device was calibrated to a predetermined optimal sensitivity before each session by tuning the coil to a designated meter reading, such that a moderate body displacement (i.e. vertical or horizontal displacement or whole trunk rotation) triggered the adjustable voltage sensor. Each triggering was scored as 1 activity unit. The apparatus was housed in a testing room which was illuminated by a 25 watt red bulb suspended 50 cm above the base of the test apparatus. Care was taken to minimize the level of extraneous noise and the temperature of the room was maintained at 30° C. Total duration of the activity test was 30 minutes. This test interval was selected in order to minimize disruptions in feeding and sleep cycles of the neonatal rat. Activity counts were automatically recorded every 10 minutes by a BRS Foringer digital system.

After the testing session, animals were weighed, ear clipped,

and tested for the appearance of physical features and reflexes (see below). Each pup was tested only once and then returned to the mother in order to maintain a constant litter size. Eight pups from at least four litters were tested at each age. Where possible, an equal number of males and females were used. All pups were weaned at 25 days of age following activity testing and housed in same-sex groups of four in wire mesh colony cages (38 x 35 x 18 cm).

Reflex and Physical Development The indices used to evaluate physical development were incisor eruption and eyelid dysjunction. The test of reflex development consisted of the startle response which measured a motor response (a jerk of the head and hind limbs) to an auditory stimulus (snap of a mousetrap placed 15 cm above and behind the rat's head) and the free-fall righting response, the ability of a rat to turn in mid-air and land on all four paws after being dropped back-downward from 35 cm.

Juvenile and Adult Behavioral Testing At both 30 and 60 days of age, six naive males and six naive females from each of the five treatment groups were tested in the open field. The open field apparatus was a wooden box (1.03 x 1.03 x 0.40 m) with an open top, white sides and floor, divided into 25 equal squares by black lines. The apparatus stood in a small windowless room. The illumination consisted of a shaded 150 watt bulb suspended 125 cm above the center of the field.

Following the suggestion of Archer (1973) for evaluating open field behavior, adaptation to this novel environment with repeated exposure served as the dependent variable. This method involved

measuring the change in frequency of occurrence of particular types of behavior over successive exposures to the open field without reference to constructs of emotionality or exploration. Four behaviors characteristic of the open field were recorded: 1) ambulation--the number of squares traversed by all four paws of a rat, 2) rearing--the number of times the rat rose on its hind paws, 3) defecation--the number of fecal boli deposited and 4) grooming--the number of seconds the rat spent washing its face or other parts of its body. Grooming time was recorded with a stopwatch to the closest second.

All animals were given three 3-minute sessions with an inter-session interval of 60 seconds. This short interval was chosen to maximize the probability of obtaining adaptation behavior in the younger animals. At the start of each session, the rat was placed in the center square of the field and allowed to explore for three minutes; measures were recorded on a minute to minute basis. At the end of three minutes, the rat was removed and the floor and sides were wiped clean with a dilute solution of Mikro-Quat to remove odor trails. At the end of the third session, the rats were weighed, ear clipped and returned to their home cages. Each animal was tested at only one age (i.e. either 30 or 60 days). All behavioral testing was conducted during the rats' dark cycle, 0800 to 2000 hours.

Statistical Analyses

A completely randomized factorial analysis of variance (Kirk, 1968) was used to examine all neonatal data. When the combination of treatments produced unequal cell frequencies, a least squares analysis

was computed. Analysis of variance was supplemented by the Newman-Keuls multiple comparison statistic for post hoc determinations (Kirk, 1968), and by t ratios for a priori analysis. The criterion for statistical significance was $p < .05$ unless otherwise indicated.

Open field data involved repeated measures on the same subject and were analyzed using a split-plot factorial analysis of variance. Conservative, rather than conventional, degrees of freedom were used to determine the significance level of the F statistic. The alpha level for between and within subject variables as well as Newman-Keuls comparisons was set at .05 unless otherwise stated.

RESULTS

Infant Activity

Fig. 1 shows total mean activity counts recorded during a 30 minute period for 1-30 day old offspring from the prenatal stress and control groups. The results of the initial analysis of variance on these data are presented in Table 1. (All statistical tables appear in Appendix A). Both main effects of Stress and Age and the Stress x Age interaction were significant ($F = 4.72$, $df = 4/55$, $p < .001$; $F = 21.68$, $df = 4/55$, $p < .001$; $F = 1.79$, $df = 48/55$, $p < .002$, respectively). The complexity of these data and the considerable amount of variation generated by the use of a completely randomized factorial design made their interpretation difficult. Therefore, on the basis of a priori assumptions, the pre- and post-weaning activity data were considered separately and separate analyses of variance were performed to determine if certain treatment groups could be combined. This procedure was adopted primarily to obtain the individual interaction terms which could not be computed from the initial analysis. To reduce the probability of obtaining spurious significance, the alpha level was decreased to .025. Table 2 lists the results of these analyses.

The control groups were pooled, since the mean activity levels of C-F pups were not significantly different from those of C-NF pups for ages 1-25. The interaction term was also non-significant. For further analysis, data from these two groups were combined and designated C-NF + C-F. On the basis of a comparison of the mean activity levels of H and ES offspring, which also revealed no significant differences,

Figure 1 Mean Total Activity Counts for N-H, H, ES, C-F and C-NF Groups for 1-30 Days of Age.

these treatment conditions were pooled to form the H + ES group. Fig. 2 shows the mean activity levels for these combined groups and the N-H group.

Preweaning Activity To determine the effects of prenatal manipulations on the mean activity levels of the pups prior to extended interaction with the foster mother, a t ratio was computed on Day 1 activity scores. At this age, pups from both the N-H and H + ES litters were significantly less active than controls ($F = 3.709$, $df = 38$, $p < .01$). The results of the analysis of variance of the total preweaning activity of the combined groups are presented in Table 3. The significant main effect of stress ($F = 11.69$, $df = 2/444$, $p < .001$) reflected the lowered activity of the offspring of N-H mothers in comparison to those from H + ES and C-NF + C-F litters. The latter 2 groups did not differ.

Simple main effects were calculated to clarify the significant Stress x Age interaction ($F = 1.79$, $df = 22/444$, $p < .01$) among the N-H, H + ES, and C-NF + C-F treatment groups. These results (Table 4), supplemented by Newman-Keuls tests, suggested that the interaction could best be explained by the fact that all 3 groups showed a change in activity during the first postpartum week. However, the increase in the control group was much more rapid and by 9 days of age their activity was significantly higher than that of N-H and H + ES pups ($p < .01$). Although levels were elevated in all groups between 11 and 17 days, peak activity seemed to occur at different ages. All groups showed a decrease in mean activity after this time. The decrease in the N-H group was more dramatic. Activity on Days 19 and 25 was significantly

Figure 2 Mean Total Activity Counts for N-H, H + ES, and C-F + C-NF Pups at 1-25 Days of Age.

less than that of the other 2 groups ($p < .05$, $p < .01$, respectively).

A parameter of primary interest was the age at which maximum activity occurred in each group. While visual inspection of Fig. 2 suggested that activity levels were highest at 9 days of age in the C-NF + C-F group, 13 days in N-H, and 17 days in the H + ES groups, statistical determination of these maxima were carried out using trend analysis and curve fitting procedures (Kirk, 1968).

The a priori prediction of a quadratic relationship between activity and age in the developing rat was confirmed by trend analysis, the results of which are listed in Table 5. Linear (N-H: $F = 11.65$, $df = 1/444$, $p < .0001$; H + ES: $F = 60.37$, $df = 1/444$, $p < .0001$; C-NF + C-F: $F = 10.54$, $df = 1/444$, $p < .001$) and quadratic ($F = 43.79$, $df = 1/444$, $p < .0001$; $F = 56.35$, $df = 1/444$, $p < .0001$; $F = 49.21$, $df = 1/44$, $p < .0001$) trend components as well as differences in linear and quadratic trend among the groups were significant ($F = 6.572 \times 10^3$, $df = 2/444$, $p < .0001$; $F = 1.852 \times 10^4$, $df = 2/444$, $p < .0001$). Since the residual values were non-significant, second degree equations were generated for each curve and the age at which maximum activity occurred was computed ($y' = -b/2a$). The results of this procedure are shown in Fig. 3. From the fitted curves, activity was highest in the N-H group at 14.2 days of age. Maximum activity occurred at 16.1 days of age in the H + ES group, while it peaked at day 14 in the combined control groups.

Postweaning Activity An analysis of the activity scores of 30 day old offspring revealed no significant differences among the groups.

Figure 3 Fitted Curves and Equations for Mean Total Activity Counts for 1-25 Day Old N-H, H + ES, and C-F + C-NF Pups. (Curves for each group have been Vincentized so that the minima of the x and y axes = 0, and the maxima = 1).

Body Weight

Body weight data obtained for pups 1-30 and 60 days of age are presented in Fig. 4 and Table 7, respectively. An analysis of variance computed on preweaning data (Days 1-25) revealed significant main effects due to Stress and Age and a significant first-order interaction ($F = 3.13$, $df = 4/408$, $p < .01$; $F = 17.08$, $df = 11/408$, $p < .0001$; $F = 1.49$, $df = 44/408$, $p < .025$). Differences were limited to the period between 17 and 25 days of age and reflected the greater body weight of pups from N-H and ES litters. Also at 21 days of age, the control non-fostered pups were significantly lighter ($p' \leq .05$) than the other four groups. The result of the analysis of variance and simple main effects are shown in Table 6.

The postweaning weights of 30 and 60 day old animals in all groups were not significantly different (Table 7). While sex differences within the groups were not apparent in the younger rats, females were significantly smaller than males at 60 days of age ($F = 78.90$, $df = 1/50$, $p < .001$).

Reflexes and Physical Features Observations on the development of reflexes and the appearance of physical features (Table 8) in the neonatal rat were rated either "no" (reflex or physical feature absent) or "yes" (reflex or physical feature present). Lord (1953) has suggested that an analysis of variance can be used with nominal data if they are distributed in certain ways. Consequently "no" and "yes" scores were assigned numerical values of 0 and 1 respectively and frequency distributions were determined. Data from each of the

Figure 4 Mean Body Weight in Grams for Pups from N-H, H, ES, C-F and C-NF Groups for Days 1-30 Postpartum.

Table 8

Development of Physical Features and Reflexes for the N-H, H, ES, C-NF, and C-F Groups During the Neonatal Period. Values Listed are the Number of Animals Showing each Variable at a Given Age. Maximum n = 8.

	Group	9	11	13	15	17	19	21
Incisors	N-H	0	1	8	8	8	8	8
	H	0	2	5	8	8	8	8
	ES	0	0	6	8	8	8	8
	C-NF	0	2	7	7	8	8	8
	C-F	0	0	8	8	8	8	8
Eyes Open	N-H	0	0	0	0	8	8	8
	H	0	0	1	1	8	8	8
	ES	0	0	0	0	6	8	8
	C-NF	0	0	0	0	7	8	8
	C-F	0	0	1	0	6	8	8
FFR	N-H	0	0	0	1	8	8	8
	H	0	0	0	0	4	8	8
	ES	0	0	0	0	5	8	8
	C-NF	0	0	0	0	4	8	8
	C-F	0	0	0	0	0	8	8
Startle	N-H	0	0	3	6	7	8	8
	H	0	6	6	8	8	7	8
	ES	0	0	8	6	8	7	8
	C-NF	0	0	8	7	7	8	8
	C-F	0	1	6	7	8	8	8

groups exhibited an L-shaped distribution which indicated that the use of analysis of variance was appropriate. The results are listed in Table 9.

Prenatal stress did not significantly retard or accelerate eye opening or incisor eruption, the former occurring in 100% of the animals on Days 17 and the latter between days 13 and 15 of age. There were likewise no significant differences among the groups in the appearance of the two reflexes examined. Free-fall righting occurred in 100% of the 17 and 19 day old pups. The development of the startle response showed greater variability with 100% levels occurring between 13 and 19 days of age.

Sexually Dimorphic Behaviour¹

Activity and body weight data from the neonatal period were further analyzed on a post hoc basis to determine whether any sex differences were present. Calculations were made on data from Days 1, 9-17, and 25. Table 10 lists the mean activity scores for males and females. The results obtained are suggestive rather than definitive because of the small cell frequencies (maximum $n = 4$), which necessitated computing multiple t tests rather than an analysis of variance. For

1. Interest in the effects of prenatal stress on sexually dimorphic behaviour stems from the work of Ward (1972). She reported that restraint stress in pregnant rats produced demasculinized male offspring. The ages from 9-17 days were of particular interest because during this neonatal period, the synthesis and steady-state levels of 5-hydroxytryptamine (5-HT), which is believed to be functionally significant in the action of gonadal hormones (Campos and Ladosky, 1972), are significantly lower in male pups than in females (Hardin, 1973a, b). Similarly transient increases in endogenous levels occur between post-natal days 10 and 15 in female rat pups (Guilian, Pohorecky and McEwen, 1973).

Table 10

Male/Female Activity Data for N-H, H, ES, C-F and C-NF Groups During the Neonatal Period. Values Listed are Total Means Counts for a 30 Minute Session.

Age	Sex	N-H		H		ES		C-F		C-NF	
		<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
1		410	-	425	370	326	689	588	488	601	501
9		837'	891'	1406	1199	1160	1153	1373	1228	1248	1155
11		1630'	1265'	1154	973	1151	1468	910	1049	1130	963
13		1346**	1072	1405	1274	947	1125**	1280	1144	1067	1498*
15		1128	1088	1091	805	1172	1270	845	883	1173	1079
17		1014	1134	1254	877	1333	1254	1142	1314	866	845
25		606	621	1009	1038	968	753	740	1118	701	730

' n=1

* p<.05

** p<.01

Table 11

Mean Weights for Males and Females from N-H, H, ES, C-F and C-NF Groups for the Neonatal, Juvenile, and Adult Periods. Values Listed are Means and Cell n's Varied from 3-6 Animals

Group		N-H		H		ES		C-F		C-NF	
Age	Sex	M	F	M	F	M	F	M	F	M	F
1		6.5	-	7.2	5.8	4.7	5.5	6.2	6.4	6.3	6.4
9		15.0	14.5	14.0	14.2	17.0	14.6	17.1	15.8	13.0	14.4
11		20.0	20.2	19.4	20.9	21.5	21.7	19.3	17.9	19.5	18.4
13		18.2	20.0	23.0	24.6	22.1	22.7	24.0	22.3	25.0	25.7
15		27.5	20.3	25.3*	19.1	22.4	23.0	23.7	27.5	20.8	25.3
17		28.5	28.0	26.0	24.8	31.3	29.6	19.4	24.8*	19.9	29.7*
25		46.7	40.0	54.6	51.7	54.7	51.7	50.1	47.5	44.7	30.9
30		71.8	69.5	69.8	54.0	63.2	58.1	56.9	69.5	66.4	57.9
60		225.9**	161.7	197.1**	168.5	209.2**	168.8	220.1**	153.8	216.2**	166.7

* n=1

* $P < .05$

** $P < .01$

certain cells, t values could not be computed because cell n was equal to 1.

Male/female activity differences occurred on Day 13 in 3 of the groups. Females were significantly more active than males at this age in both the C-NF and ES groups ($t = 2.39$, $p < .05$; $t = 15.04$, $p < .01$, respectively). In the N-H group, the reverse trend was seen ($t = 5.829$, $p < .001$).

Significant differences in weight between males and females during this period were minimal and did not coincide with differences in activity levels (Table 11).

Juvenile and Adult Open Field Behavior

The open field data were analyzed in terms of the adaptation rates of four variables which are characteristic of the rat when exposed to a novel environment. All data in this section were analyzed for overall differences in adaptation rates among the 5 treatment groups and for differences between male and female rates of adaptation. Table 12 and 13 list the results of analyses of variance from the four dependent variables for 30 and 60 days of age respectively. Analyses computed to determine the existence of within group male/female differences are shown in Table 14.

Day 30 Squares The results of an initial analysis of variance on 30 day old rat pups' ambulation scores revealed that neither of the between subject variables (Stress and Sex) were significant nor were any of the interactions which contained these variables.

The within subject factor of Trials was significant using a conservative F ($F = 47.11$, $df = 1/25$, $p < .001$). Since this indicated that

adaptation occurred with repeated exposure to a novel environment, the adaptation curve for each treatment group was determined (see Fig.5) and these were then compared. The patterns of adaptation among the stress groups and between them and the control groups were significantly different. Newman-Keuls tests indicated that both the C-NF and the C-F groups showed a significant ($p's < .01$) decrease in ambulation over all three sessions. The animals from the Handled and Electric Shock litters exhibited a significant decrease in the number of squares crossed between trials 1 and 2 ($p's < .01$) but not between 2 and 3. Ambulation rates in the Non-Handled group decreased only after the second trial ($p < .01$).

The data were collapsed across treatments to compute the adaptation rates for males and females. While females showed a significant decrease over all 3 trials ($p's < .01$), ambulation scores for males showed a significant decrease only between trials 1 and 2 ($p < .01$). Within group sex differences were not significant.

Rearing An analysis of variance revealed once again a significant decrease in rearing ($F = 39.75$, $df = 1/25$, $p < .001$). The H and ES groups again showed a similar pattern of habituation with both groups exhibiting a significant decrease in rearing behavior over all 3 trials ($p's \leq .05$). These two groups again differed from the Non-Handled group, which showed a significant decrease only between trials 2 and 3 ($p < .01$). However, while the N-H group differed from the C-NF group, the H and ES groups did not. The animals from the fostered control litters differed from their non-fostered controls, with the former exhibiting decreased

Figure 5 Adaptation Rates for Four Open Field Variables Obtained at 30 and 60 Days of Age from N-H, H, ES, C-F and C-NP Pups. Values Shown are Means and Data have been Collapsed Across Sex.

rearing behavior only between trials 1 and 2 ($p < .01$). Adaptation rates for both males and females declined significantly ($p's < .01$) from trials 1-3. Statistically significant group differences were also not evident.

Grooming There was no change in the amount of time spent grooming over the 3 trials for any of the groups.

Boli Only the number of fecal boli deposited during the first-3-minute session was analyzed. A significant Sex x Minute interaction ($F = 5.62$, $df = 1/25$, $p < .05$) was found. Boli counts decreased in males over the 3 minute period; for females there was an increase from minute 1 to 2 and then a decline.

Day 60 Squares At 60 days of age the only significant main effect was Trials ($F = 62.6$, $df = 1/25$, $p < .001$) The two control groups again showed similar patterns of adaptation but differed from the 3 stress groups, which in turn differed from each other. However, the pattern of adaptation had changed within each group with age. The control groups now showed a significant decrease ($p's < .05$) in number of squares crossed only after the second trial. The H and ES groups exhibited a significant decrement ($p's < .05$) in activity over all 3 trials, the ambulation rate for the N-H group decreased between trials 1 and 2 but not between 2 and 3.

The male/female difference in habituation rates no longer existed at 60 days of age. Both sexes showed a significant decrease ($p's < .01$) over the 3 trials, a pattern which was characteristic of the females at 30 days of age but not of the males. However, there was a trend for females in all groups to be more active in the open field than males.

Rearing A breakdown of the significant Trials effect ($F = 132.40$, $df = 1/25$, $p < .0001$) by a Newman-Keuls test indicated that the adaptation rates for the H, ES, C-F, and C-NF groups were similar, showing a significant decline (p 's $< .01$) over all trials. The N-H group showed a decrease only between session 1 and 2 ($p < .01$). Adaptation rates for males and females were identical, showing a significant decrease (p 's $< .01$) over all 3 trials.

A significant main effect due to prenatal stress ($F = 3.51$, $df = 4/30$, $p < .01$) reflected the greater rearing behavior ($p < .01$) of the C-F group than that of the C-NF group and that of the stress groups (p 's $< .01$). The ES pups reared significantly less (p 's $< .01$) than the N-H and H groups.

Grooming At 60 days of age an overall analysis of variance revealed a significant Trials effect ($F = 7.31$, $df = 1/25$, $p < .05$). However, an analysis of the data for each treatment group did not indicate any adaptation over trials. Further analysis of the data indicated that the significant Trials effect reflected the increase in grooming over trials exhibited by the males ($F = 7.54$, $df = 1/25$, $p < .05$) but not for females (Table 14).

Boli During the first 3-minute session in the open field, males defecated significantly more than females ($F = 10.95$, $df = 1/50$, $p < .002$). However, in both sexes boli counts decreased over the 3-minute trial ($F = 7.31$, $df = 1/25$, $p < .05$).

In an effort to integrate the open field data from this study

Table 15

Open Field Data for Trial 1 for 30 and 60 Day Old PMS and Control Pups.
Values Shown are Means for a 3 Minute Period

		<u>Age 30</u>	<u>Age 60</u>
<u>Squares</u> (Number)	N-H	195	261
	H	217	310
	ES	316	285
	C-NF	245	258
	C-F	243	335
<u>Rearing</u> (Number)	N-H	96	133
	H	97	131
	ES	115	93
	C-NF	138	128
	C-F	129	181
<u>Grooming</u> (Seconds)	N-H	118	52
	H	73	39
	ES	106	25
	C-NF	51	36
	C-F	78	28

with those of others who have evaluated open field behavior after prenatal stress, data from the first 3 minute trial were separately analyzed for both age groups. These data are presented in Table 15 and the analyses in Tables 16 and 17.

Squares Ambulation scores for the ES animals were significantly elevated over those for the other four groups at 30 days of age ($F = 3.18$, $df = 4/55$, $p < .02$). Individual activity levels of the fostered and non-fostered control groups were not statistically different from one another and were significantly higher than those of both the H and N-H groups (p 's $< .01$). Between these latter two groups, ambulation scores of the H group were significantly higher ($p < .01$). At 60 days of age, these differences were no longer observed. The number of squares crossed decreased significantly during the first 3-minute session at both ages ($F = 90.25$, $df = 1/55$, $p < .001$; $F = 24.00$, $df = 1/55$, $p < .001$).

Rearing Prenatal stress did not significantly influence rearing behavior at 30 days of age. In 60 day old animals, a significant Stress effect ($F = 4.19$, $df = 4/55$, $p < .005$) reflected the greater frequency of rearing exhibited by the C-F group and the low frequency of rearing in the ES group when compared with the other groups (p 's $< .01$).

Grooming The amount of time spent grooming by the animals in the various treatment groups was not significantly different at either age.

DISCUSSION

The general findings of this experiment were that the ontogeny of locomotor activity is vulnerable to prenatal manipulations and that these manipulations alter ambulation scores as well as the adaptation rates of several variables in the open field.

Handling or the administration of electric shock to the pregnant animals delayed by 2 days the age at which maximum activity occurred in the offspring and lowered the activity levels of these animals during the neonatal period. The fact that litters from Non-Handled mothers also showed lower activity during this period (although age of peak activity was not altered) suggests that the vaginal smearing procedure per se constituted a stressful situation. The offspring of these females may be considered as having been exposed to early prenatal stress.² It would appear that the trimester during which stress is applied rather than the precise nature of the stressor may determine offspring effects of PMS.

The fostering of pups at birth did not significantly affect the ontogeny of locomotor activity. Significant differences were not found between fostered and non-fostered control groups either at Day 1 or throughout the neonatal period. However, this does not eliminate the possibility that this postnatal manipulation could interact with

2. Smears were taken every second day. Therefore, the females could have been up to 46 hours pregnant at the time of their last smear.

prenatal stress to attenuate the effects produced by the latter. Moreover, it would appear that certain environmental manipulations do attenuate the effects of PMS. The differences between groups observed during the preweaning period (i.e., 1-25 days of age) were not observed at 30 days of age. Weaning and group housing have been shown to minimize if not eliminate differences in open field behavior and the reaction to handling between prenatal stress and control offspring (Ader, 1965; Archer, 1970).

Prenatal stress did not produce a generalized retardation in development. Differences in body weight among the control and stress groups were transient. Moreover, the appearance of physical features and reflexive behavior was not retarded in the prenatal stress groups.

Campbell and his colleagues have suggested that the age-related changes in locomotor activity parallel the ontogeny of brain arousal mechanisms (Campbell et al., 1969; Moorcroft et al., 1971; Campbell and Mabry, 1972) and reflect the caudal-rostral sequence of CNS development. The maturation of hindbrain excitatory centers, they argue, is responsible for the sharp increase in activity that occurs between 10 and 15 days postpartum. The decrease in activity seen after this time is due to the development of cortical inhibitory centers.

Campbell et al. (1969) have purported to have measured the ontogeny of behavioral arousal that is mediated by endogenous stimulation from maturing structures in the CNS, developing endocrine systems and sensory/motor inputs. However, activity testing in the present experiment most likely also measured the neonates' reactivity to the

exogenous stimulation involved in testing. The short test session (30 minutes) probably did not provide the animal with adequate opportunity to adapt to handling, separation from the mother, and exposure to a novel environment. Support for this interpretation comes from recent evidence that the neonatal rat, contrary to what had previously been reported (i.e. Schapiro, 1968), is not unresponsive to external stimuli. Rather, the duration of the stress response is extended in the young organism (Zarrow, 1966; Hiroshige, Sata, and Abe, 1971).

Whether the present activity measure in the neonatal rat primarily reflected responsiveness to external stimuli, responsiveness to internal stimuli, or responsiveness to both, can only be speculated on. Perhaps the activity scores from 1 to 9-10 days of age primarily reflected the neonatal rats' reactivity to external stimulation. During the second and third week postpartum when brain development is rapid (Himwich, 1962; Flexner, 1965) and sensory stimulation from developing systems increases reticular activation, the response to endogenous stimulation may have predominated.

Two qualitatively different prenatal stressors (i.e. handling and electric foot shock) altered the reactivity/arousal pattern and overall levels of locomotor activity in the same manner. This is not totally unexpected in that these stress procedures produce certain common hormonal changes (Archer and Blackman, 1971) and it is presumably through these hormonal alterations that the effects of PMS are mediated. A variety of prenatal stressors such as handling

(Ader and Conklin, 1963; Ader and Plaut, 1968), conditioned avoidance (Thompson, 1957; Ader and Belfer, 1962; Thompson and Quinby, 1964; Morra, 1965), crowding (Keeley, 1962), and swimming and tilting (DeFries et al., 1964, 1967) have been shown to all decrease open field activity in the adult rat.

Joffe (1965) has pointed out that certain techniques, among them electric shock, used in prenatal stress studies could directly affect the fetus. The similarity of the data from the Handled and Electric Shock groups does not support this hypothesis. Although possible, it is highly improbable that the handling procedure employed in this experiment could directly influence the fetus.

Open Field

Ambulation scores during the first 3-minute open field exposure of the N-H and H groups were significantly lower than those of C-NF pups. These findings are consistent with the reports of decreased ambulation in the open field for prenatal-stress rats generally reported by most investigators (See Archer and Blackman, 1971). That the ES animals were significantly more active than controls might be due to the fact that the number of shocks these animals received was significantly higher than those reported by other workers using a conditioned avoidance procedure (Thompson and Quinby, 1964), or the added unpredictability and inescapability of the shock might also have significantly increased its stressfulness (Weiss, 1972). Ressler and Anderson (1973a, b) have reported bidirectional responses in the offspring as a function of the number of shocks their mothers had received prior to

ming. Contrary to what has previously been reported, no correlation, either inverse or direct, was found between changes in ambulation and defecation scores.

The difference in the number of squares crossed between the control and PMS groups was more pronounced at 30 days of age and is also consistent with the studies of DeFries and Weir (1964) and Ader and Plaut (1968). They found that the behavioural effects of PMS were most apparent at an early age (i.e. 40 days) and that these differences tend to wane in older animals (i.e. 90-120 days) unless the offspring were first tested at an early age. Each animal in this study was tested only once.

The traditional interpretation of these decreased ambulation scores is that PMS increases the emotionality of stressed offspring. However in a recent review of the literature, Archer (1973) has pointed out that owing to the occurrence of both immobility and escape behavior as alternate forms of emotional behavior, ambulation shows little descriptive validity as an indication of emotional responsiveness in the rat. Alternatively he has suggested the use of adaptation rates to measure response to a novel environment without assuming that they measure unitary motivational constructs. In the rodent, ambulation and defecation in a novel environment decrease with repeated exposure while rearing and grooming increase. The more rapid the change over sessions, the higher the adaptation rate. It is therefore possible that the decreased open field activity of PMS pups might reflect differences in adaptation rates for ambulation between control and stressed pups with the latter showing a continued decrement upon repeated exposure to a novel environment. At 30 days of age this was not the case.

Ambulation scores decreased significantly over all three trials in the C-NF and C-F groups but did not for N-H, H, and ES pups. However, adaptation rates for ambulation at 60 days of age support this type of interpretation in that H and ES offspring showed a decrease across all 3 trials while the 2 control groups did not.

In general agreement with the normative data reported by Bronstein (1972a, b), the pattern of adaptation for ambulation also changed as a function of age in the control groups. In the C-NF and C-F groups, there was a continual decrease in ambulation across trials for the 30 day old animals while adaptation rates in the 60 day old rats stabilized after the initial exploratory period.

The adaptation rates of the 4 open field variables were differentially sensitive to the various manipulations. Adaptation rates for ambulation differentiated among the PMS groups, and between these and the control groups at both ages. Rearing was mainly affected by post-natal manipulations. Adaptation rates and overall levels of rearing differed at 30 and 60 days respectively for the fostered and non-fostered controls.

There were several differences between the effects of PMS on neonatal and adult behavior. In the neonate, all 3 prenatal stressors reduced the offsprings' activity/reactivity. However in the adult offspring, the direction of the change in reactivity seemed to depend upon the severity of the stress. Vaginal smears and handling of the mothers decreased open field ambulation. Offspring of ES mothers showed an increased open field activity. During the neonatal period

there were no significant differences between control fostered and non-fostered offspring. However, open field rearing behavior was sensitive to this postnatal manipulation and differentiated between C-NF and C-F pups.

EXPERIMENT II

The design of Exp. I, in which subjects were tested only once for neonatal locomotor arousal, may not have been suitable for determining the ontogeny of behavioral arousal mechanisms. Activity measures obtained using this design probably primarily reflected the animal's reactivity to the external stimulation produced by the testing procedure rather than endogenous brain arousal processes. To eliminate or attenuate this contribution, a repeated test design was used in Exp. II. There is evidence to suggest that habituation does occur in the neonatal rat with repeated exposure to a stimulus (Ashida, 1972; Feigley, Parson, Hamilton, and Spear, 1972) although the rate of habituation is inversely related to age. The use of the more economical repeated test design in this experiment also permitted the examination of several other independent variables which have previously been shown to interact with prenatal stress to alter either the direction or intensity of the offspring's behavioral response. Because primary interest was in the development of behavior, Exp. II focused on the effects of these variables from 1-30 days postpartum.

Since the data from Exp. I suggested that the trimester during which stress was administered could influence the extent to which offspring's behavior was altered, pregnant females were exposed here to stress at one of two different gestational periods, days 6-12 or 15-21 postmating. Electric foot shock was used exclusively as a prenatal stressor because the results of the previous experiment indicated that

two qualitatively different stresses, handling and electric shock, produced similar behavioral and physical changes in pups during the neonatal period.

In the previous experiment all PMS pups were cross-fostered at birth to non-stressed mothers. Joffe (1969) has pointed out that this procedure can produce false negative results and the divergent results reported for adult PMS offspring support this idea. Thompson and his colleagues (Thompson et al., 1963; Thompson and Quinby, 1964) found no differences in the behavior of cross-fostered and non-fostered animals when tested as adults in the open field. However Hockman (1961) reported that the decrease in open field activity occurred only in PMS pups that had been fostered or cross-fostered; there was no significant decrease in the activity of experimental animals reared by their own mothers. An interaction between prenatal stress conditions, cross-fostering and sex was found by Ader and Conklin (1963). Female offspring from the experimental group showed significantly shorter latencies than control offspring only if they were raised by their own mothers; male treated offspring showed altered behavior only if they were fostered. In the following experiment, half of the stressed litters were cross-fostered at birth to non-stressed control dams. The remainder were left with their stressed mothers.

This experiment was also designed to test whether the sex of the offspring would interact with prenatal stress to produce dimorphic effects. Sex differences in response to prenatal stress have been reported for adult offspring (Thompson et al., 1962; Havlena and

Werboff, 1973; Thompson and Quinby, 1964; Ader and Plaut, 1968; Corson et al., 1970) and it was of interest to determine whether these directional differences would occur during the neonatal period particularly around 12-15 days postpartum, when there is a transient increase in ovarian activity (Presl, Herzmann, and Horsky, 1969).

Whether prenatal maternal stress would alter the ontogeny of natural behaviors other than locomotor activity was also examined. Spontaneous alternation (SA) was chosen because it exhibits a distinct ontogenetic pattern (Douglas et al., 1973; Egger, 1973a, b; Egger et al., 1973). It consists of the tendency of an adult animal on two consecutive unrewarded trials in a T-maze, to enter one goal arm and then the other. However, the pre-weanling rat (i.e. 15-17 days of age) will choose the same goal arm on both occasions (perseverative behavior). Chance levels of alternation are seen at approximately 22 days of age. Alternation behavior reaches adult levels between 28 and 30 days postpartum.

Experiment II also investigated the effects of PMS on the development of endogenous levels of norepinephrine (NE), serotonin (5-HT) and its major catabolite 5-hydroxyindole acetic acid (5-HIAA). The ontogeny of behavioral arousal and SA has been linked with the maturation of these amine systems. Campbell and his associates have correlated the hyperarousal seen in 15 day old rats with the maturation of noradrenergic hindbrain centers and the transient weakness of telencephalic serotonergic and cholinergic systems. They state that the capacity of the serotonergic system to inhibit behavioral arousal reflects

the more caudal location of 5-HT containing cell bodies in the mid-brain raphe system.

A similar hypothesis of synergistic action between serotonergic and cholinergic systems has been postulated for the maintenance of SA (Swonger and Rech, 1972). They hypothesized that a raphe based serotonergic system acts as a gain controller for limbic based cholinergic inhibitory processes, effecting an increase in inhibitory tone in response to an increase in arousal state. If the ontogeny of behaviour can serve as an index of CNS maturation, then alterations in the activity level and age at which peak activity or percentage of alternation behaviour occurs should reflect variations in steady state levels of 5-HT, 5-HIAA, and NE. The results of Exp. I indicated that PMS affects the development of locomotor activity. This leads to the prediction that brain development would be altered and hence amine levels affected in rats subjected to PMS.

PROCEDURE

Subjects

Nulliparous female Wistar rats (225-250 grams) from Bio-Breeding Labs, Ottawa, were used. They were mated to Wistar males in the ratio of 3:2, 48 hours after delivery to the laboratory. Vaginal smears were not used to determine the pregnancy status of the females. They were simply left with the males for 5 days. After this time, the females were removed and randomly assigned to one of 2 prenatal stress conditions or to the control group. All females were housed individually in polyethylene cages with ad libitum water and Purina rat chow. They were maintained on a reversed 12 hours light-dark cycle with lights off at 0800 hours.

Stress Procedures

Fifteen unsignalled, inescapable foot shocks (0.5 mA for 0.5 seconds) served as the stressor. The apparatus and shock source were identical to those used in Exp. I. Electric shock was administered during a 30 minute test session to animals in both the Early and Late prenatal stress groups.

Group I Early Prenatal Stress (EPS) The females assigned to this group were exposed to shock stress for the 7 consecutive days immediately following removal from the males. At birth, half of the litters in this group were cross-fostered to non-stressed mothers and the remainder were raised by their biological mothers.

Group II Late Prenatal Stress (LPS) Fifteen days after mating, shock stress was administered to females in the LPS condition for 7 consecutive days. Half of the litters were cross-fostered at birth to non-stress females and the rest were left with their natural mothers.

Group III Control (C) These females were not disturbed during the gestation period. At birth half of the litters were fostered to control females and the others were not.

Four replications were necessary to complete the experiment. Of the 24 females in the first 3 replications, 6 were randomly assigned to each of the 2 stress conditions and the remaining 12 were designated as either control or foster mothers. In the fourth replication, 10 females were assigned to the EPS conditions, 4 to the LPS group, and 10 served as control or foster mothers.

Offspring Testing

Infant Activity Six male and six female pups from fostered and non-fostered treatment groups were tested at 1, 3, 6, 9, 12, 15, 18, 21, 25, and 30 days of age. All animals were tested at each age and each group consisted of the offspring from at least 3 litters.

As in Exps. I and II, activity was recorded during a 30 minute test session with the field capacitance movement sensing device. The size of the cardboard container in which pups were placed during testing varied with the age of the animal: Day 1, 3, and 6—7.5 x 7.5 x 5 cm; Day 9, 12, and 15—8.7 x 10.0 x 15 cm; Days 18, 21, 25, and 30—11.0 x 9.0 x 15 cm. The apparatus was housed in a 64 x 64 x 57 cm sound-attenuated chamber. A fan mounted outside the box provided

ventilation and a low masking noise. A 100 watt red floodlight (General Electric) suspended 55 cm above the movement sensor served as the light and heat source. The temperature was maintained at 35-36°C.

Reflex and Physical Development Eye opening, incisor eruption, the startle response and the free-fall righting reflex were scored for each rat immediately after activity testing.

Spontaneous Alternation Alternation behavior was recorded at 17, 22, 27, 32 and 37 days of age. Six males and six females not previously tested, from at least 3 litters within each fostered and non-fostered treatment condition were tested at all 5 ages.

Testing was conducted in a grey plywood T maze. A sectioned hinged plexiglas lid permitted separate access to the start box, the main alley and the 2 goal arms. The main alley measured 11.5 x 35.0 x 11.5 cm. A 15 cm section of this alley served as the start box and was separated by a black guillotine door. The goal arms measured 11.5 x 25.0 x 11.5 cm, with 9 cm diameter holes providing access to these arms and black plastic guillotine doors separating the goal alleys from the main alley.

The maze was housed in a small windowless room maintained at a constant temperature of 22°C. A shaded 25 watt red bulb suspended 25 cm above the choice point of the maze, provided the only illumination.

Each alternation test consisted of two trials. A trial consisted of placing the animal in the start box for 10 seconds. The guillotine door was then raised and the rat was given 5 minutes

in which to enter one of the goal arms with all 4 paws. The rat was then left in the chosen arm for 50 seconds after which it was removed to a holding cage for 60 seconds. During this inter-trial-interval the maze was wiped with a solution of Micro-Quat and sprayed with Lysol deodorizer to eliminate odor trails. Alternation behavior was scored twice daily, with a four hour interval between tests. This procedure was adopted to insure the independence of results from each test (Dember and Fowler, 1958).

Biochemical Determinations A fluorometric assay technique was used to determine the endogenous levels of serotonin, 5-hydroxyindoleacetic acid, and norepinephrine in brain tissue. The assay technique was modified by Peters (personal communication) from the methods of Miller, Cox, Snodgrass, and Maickel (1970) and Curzon and Green (1970). The details of this procedure are presented in Appendix B.

Rats that had not undergone behavioral testing were sacrificed by decapitation at 1, 12, and 25 days of age. The brains were quickly removed and dissected on an ice-cooled, saline rinsed plate. Whole brains were first divided in half by a sagittal section along the midline suture. The cerebral and cerebellar cortices were removed from one half and the remaining brain stem tissue was used for biochemical analysis. Tissue from 12 pups from each of the 6 groups were assayed at each age, with an equal number of males and females in each group.

Statistical Analyses A split-plot factorial analysis of variance was used to evaluate neonatal activity and body weight data. The Newman-Keuls statistic was used for post hoc comparisons. As in the previous

experiment, reflex and physical features data were coded and analyzed by a split-plot factorial analysis of variance.

Spontaneous alternation data were first analyzed for position preference in order to determine the a priori probability of alternation for each treatment group (Dember and Fowler, 1958). The probability of alternation is equal to $1 - (\text{percent of responses to preferred side}^2 + \text{percent of responses to non-preferred side}^2)$. This value was then used in the Chi Square statistic to determine whether the alternation behavior was statistically different from chance at a particular age.

The within subject design necessitated the use of a statistic other than Chi Square to determine the pattern of alternation behavior over the 5 test days. Therefore, SA data for each subject for each day were given a score of 0, 1, or 2; 0 = no alternation behavior on a given day, 1 = alternation on either the first or second test, 2 = alternation on both tests. Data were then analyzed using a split-plot factorial design. The small number of animals in each group (12) did not make it feasible to examine the data for sex differences. However, the number of males and females in each group was equal.

Biochemical data was subjected to a multivariate analysis of variance. Cooley and Lohnes (1971) have suggested the use of this statistic when dependent variables are correlated as is the case with brain serotonin, 5-hydroxyindoleacetic acid, and norepinephrine.

RESULTS

Neonatal Activity

Figures 6a and b show the mean activity levels of male and female non-fostered and fostered offspring of C, EPS, and LPS mothers for days 1-30 postpartum. The results of the initial analysis of variance are listed in Table 18. Neither the main effect of Sex nor any of the interactions containing this variable was significant and this factor was not considered in subsequent analyses. While both Fostering and Stress per se did not significantly alter activity, the Age x Stress and the Age x Stress x Fostering interactions were significant ($F = 13.85$, $df = 2/60$, $p < .001$; $F = 3.70$, $df = 2/60$, $p < .05$). Activity also varied with age ($F = 16.02$, $df = 1/60$, $p < .001$) (Table 18).

Fostering vs Non-Fostering Activity data from C, EPS, and LPS offspring were analyzed separately to determine the effects of fostering versus non-fostering on pups from the 3 treatment groups. The results of these analyses (Table 19) indicated that the overall activity levels of the non-fostered and fostered pups were not significantly different in any of the groups. However, the Age x Foster interaction was significant for the non-fostered and fostered control groups ($F = 4.966$, $df = 1/23$, $p < .05$) and reflected primarily the different ages at which peak activity occurred. A visual inspection of the data indicated that in the non-fostered control pups, activity was maximal at 18 days of age. Peak activity occurred 3 days later when control pups were fostered (see Fig. 7). The curves were similar in every other respect.

Figure 6a Mean Total Activity Counts for Non-Fostered Male and Female Pups from EPS, LPS and C Litters at 1-30 Days of Age.

Figure 6b Mean Total Activity Counts for Fostered Male and Female Pups from EPS, LPS, and C Litters at 1-30 Days of Age.

Figure 7 Mean Total Activity Counts for Fostered and Non-Fostered Early Prenatal Stress (Upper), Late Prenatal Stress (Middle) and Control (Lower) Offspring at 1-30 Days of Age.

The fostering of pups did not alter activity levels at either 1 or 30 days of age (Table 20).

Stress Effect The breakdown of the significant main effect of Stress ($F = 3.78$, $df = 2/66$, $p < .02$) indicated that the Day 1 activity of the pups from the fostered and non-fostered control litters was significantly lower ($p < .05$) than that of the 2 EPS groups. Neither the main effect of Fostering nor the Stress x Fostering interaction was significant.

As previously mentioned the initial analysis of variance on mean activity for 1-30 day old pups revealed a significant Age x Stress x Fostering interaction. Because the first order interaction involving Age and Stress was also significant, the fostering variable was factored out and the data from the 3 non-fostered and the 3 fostered groups were considered separately.

Non-Fostered Pups The results of an analysis of variance on data (i.e., Days 1-) for the 3 non-fostered groups are listed in Table 21. The main effect of Stress was not significant. The Age main effect and the Age x Stress interaction were significant ($F = 6.979$, $df = 1/33$, $p < .05$; $F = 3.36$, $df = 2/33$, $p < .05$ respectively). Fig. 8 shows the relationship between these variables. The results of a simple main effects (Table 22) supplemented by Newman-Keuls tests indicated that the interaction reflected the difference between the control non-fostered pups and offspring of EPS and LPS; the latter 2 groups differed only at 6 days of age ($p < .05$). Activity of pups in the prenatal stress groups increased and remained elevated above control levels during the first two weeks postpartum ($p's < .05$ and $< .01$), after which it gradually

Figure 8 Mean Total Activity Counts for Non-Fostered EPS, LPS and C Pups (Upper), and Fostered EPS, LPS and C Pups (Lower) at 1-30 Days of Age. Data Shown are Collapsed Across Sex.

declined. In comparison, control offsprings' activity remained relatively unchanged between 3 and 15 days of age, after which time it rapidly increased and then sharply declined. Activity levels at 25 and 30 days of age were similar for all 3 groups.

Trend analysis and curve-fitting procedures, the results of which are listed in Table 23 and Fig. 9 respectively, were utilized to determine the age at which maximum activity occurred. Linear (EPS: $F = 4.41$, $df = 1/264$, $p < .05$; LPS: $F = 8.87$, $df = 1/264$, $p < .01$; C: $F = 20.77$, $df = 1/264$, $p < .01$) and quadratic (EPS: $F = 9.88$, $df = 1/264$, $p < .01$; LPS: $F = 20.35$, $df = 1/264$, $p < .01$; C: $F = 20.77$, $df = 1/264$, $p < .01$) trend components as well as the differences in linear trend among the groups were significant ($F = 16.695$, $df = 2/264$, $p < .01$). There were no significant differences in quadratic trend components among the groups. Since residual values were not significant, second degree equations were generated for each curve and the age at which maximum activity occurred was determined.

The results of the curve-fitting procedure indicated that maximum activity occurred in the non-fostered EPS and LPS pups at 10.1 and 10.0 days of age respectively, but not until 16.8 days of age in the control non-fostered pups.

Fostered Pups The results of an analysis of variance of the preweaning activity data for C, EPS and LPS fostered pups were similar to those obtained for the non-fostered pups in that the main effect of Age and the Age x Stress interaction were significant ($F = 10.963$, $df = 1/33$, $p < .001$; $F = 17.406$, $df = 2/33$, $p < .001$ respectively). Examination of

Figure 9 Fitted Curves and Equations for Mean Total Activity Counts for Non-Fostered EPS, LPS and C Pups at 1-25 Days of Age. (Curves for each group have been Vincentized so that the minima of the x and y axes = 0, and the maxima = 1).

Figure 10 Fitted Curves and Equations of Mean Total Activity Counts for 1-25 Day Old Fostered Pups from EPS, LPS and C Litters. (Curves for each group have been Vincentized so that the minima of the x and y axes = 0, and the maxima = 1).

the interaction term using simple main effects (Table 24) indicated that all 3 fostered groups showed a different pattern of activity during the preweaning period. As was observed for non-fostered controls, activity of the fostered controls during the first two weeks after birth was low, increased sharply at 21 days of age, and decline between 21 and 25 days of age. While the activity in both of the prenatal stress groups was again significantly ($p's < .01$) elevated over control levels in the period between 1-15 days of age, peak activity appeared to occur at different ages in these two groups (Fig. 8). The results of a trend analysis and curve-fitting confirmed this observation (see Table 25 and Fig. 10 respectively). The EPS and LPS pups exhibited maximum activity levels at 8.6 and 7.7 days of age respectively, which was substantially earlier than the 19.3 days of age observed in the control fostered pups. At 25 days of age, activity levels of the 3 groups were not significantly different.

Postweaning Activity An analysis of the activity scores of 30 day old offspring (Table 20) did not reveal significant differences among any of the non-fostered stressed, fostered stressed and control groups.

Body Weight

Body weight data obtained for 1-30 day old pups are presented in Fig. 11. An analysis of variance on these data indicated that neither the sex of the pups nor the postnatal rearing conditions had any significant effect on body weight during this period. Only the main effects of Stress and Age and the first order interaction involving these two variables were significant ($F = 3.59$, $df = 2/60$, $p < .03$;

Figure 11 Mean Body Weight Data in Grams for 1-30 Day Old EPS, LPS and C Pups. Data are Collapsed Across Sex.

$F = 3,738.286$, $df = 1/60$, $p < .001$; $F = 3.62$, $df = 2/60$, $p < .05$ respectively). However, a simple main effects analysis showed that differences among the groups were minimal. The control groups were significantly lighter than both the EPS ($p < .05$) and LPS ($p < .05$) groups at 6 days of age. At 21 days of age, LPS ($p < .05$) pups were heavier than the other 2 groups. At 30 days of age, control pups were significantly heavier than EPS ($p < .05$) offspring, while the LPS and control offspring did not differ. The results of the analysis and the simple main effects are listed in Tables 26 and 27 respectively.

Physical Features and Reflexes Table 28 lists the number of animals in each group exhibiting the physical features and reflexes scored during the neonatal period. As in the previous experiments, observations were assigned numerical values of either 0 or 1 and analyzed with an analysis of variance. The results are presented in Table 29.

The incidence of eye-opening and incisor eruption increased with age in all 6 groups ($F = 239.99$, $df = 1/60$, $p < .001$). The main effects of Stress and Fostering as well as the interactions containing these terms were not significant. Eye-opening occurred in 100% of the pups in the fostered and non-fostered stress and control groups at 18 days of age while incisors were apparent in all animals by day 15.

Similarly, there was an increase in the number of animals in whom the startle response and the free-fall righting reflex could be elicited from Day 9 to Day 11. The Age x Stress interaction was not significant when the F statistic was tested with conservative degrees of freedom ($F = 2.27$, $df = 2/66$, $p < .08$) although there was a tendency

Table 28

The Development of Physical Features and Reflexes for EPS, LPS, and Control Fostered and Non-Fostered Groups. Values Given are the Number of Animals Showing Each Variable at a Given Age. Maximum=12

	<u>Group</u>	<u>9</u>	<u>12</u>	<u>15</u>	<u>18</u>	<u>21</u>
Eyes Open	EPS-NF	0	2	2	12	12
	EPS-F	0	0	3	12	12
	LPS-NF	0	0	6	12	12
	LPS-F	0	0	4	12	12
	C-NF	0	0	0	12	12
	C-F	0	0	0	12	12
Incisors	EPS-NF	0	7	12	12	12
	EPS-F	3	11	12	12	12
	LPS-NF	0	12	12	12	12
	LPS-F	0	11	12	12	12
	C-NF	0	8	12	12	12
	C-F	0	11	12	12	12
FFR	EPS-NF	0	0	1	10	12
	EPS-F	0	0	0	12	12
	LPS-NF	0	0	0	11	12
	LPS-F	0	0	0	12	12
	C-NF	0	0	0	12	12
	C-F	0	0	0	12	12
Startle	EPS-NF	0	8	12	12	12
	EPS-F	0	6	12	12	12
	LPS-NF	0	8	12	12	12
	LPS-F	0	10	11	12	12
	C-NF	0	4	11	12	12
	C-F	1	4	11	12	12

for the animals in EPS and LPS groups to show the startle response at an earlier age in comparison to the control group. Maturation of the free-fall righting reflex occurred at 18 days of age in all of the groups, while the startle response was seen in 100% of the animals between 15 and 18 days of age.

Spontaneous Alternation Alternation rates for all 6 groups at 17, 22, 27, 32, 37 days of age are presented in Fig. 12. No significant trial 1 direction preferences were found. Using trial 1 data, expected rates of alternation were calculated for each group at each age using the formula of Dember and Fowler (1958). The difference between these and observed rates were tested using the Chi-Square statistic. The results are indicated on Fig. 12. Alternation behaviour did not consistently increase with age from perseveration to chance levels to alternation in any of the groups. However, while 17 day old animals showed perseverative behaviour or alternated at chance levels, at 37 days of age, animals in 5 of the 6 groups were alternating at a reliable rate of approximately 65%.

Analysis of variance performed on the assigned numerical alternation scores showed no significant main effects or interactions between the Stress, Age, and Fostering variables (see Table 30).

Biochemical Assays The results of fluorometric determination of endogenous levels of serotonin (5-HT), norepinephrine (NE) and 5-hydroxyindoleacetic acid (5-HIAA) in the "brain stems" of 1, 12, and 25 day old control and stress pups are listed in Table 31. The data were analyzed using a multivariate F statistic (Kramer and Jensen, 1969)

Figure 12 Spontaneous Alternation Data for Fostered and Non-Fostered Pups from EPS, LPS and C Litters at 17-37 Days of Age. Values Listed are the Percent of Animals Exhibiting Alternation Behavior. Letters Indicate Perseverative Behavior (P), Chance Levels (C) or Reliable Levels (A) of Alternation.

and adopting the suggestion of Hummel and Sligo (1971), a univariate analysis of variance was performed for each variable only if the multivariate statistic was significant. The results of the multivariate test for 4 independent and 3 dependent variables are listed in Table 32. Age was the only independent variable for which the multivariate test was significant ($\lambda = .3383$, $df = 2/180$, $D.V. = 3$, $p < .001$). The univariate F tests for serotonin ($F = 112.87$, $df = 2/180$, $p < .001$), norepinephrine ($F = 102.09$, $df = 2/180$, $p < .001$) and 5-HIAA ($F = 75.19$, $df = 2/180$, $p < .001$) were also significant and reflected significant increases in the endogenous levels of the 2 amines and 5-HIAA in stress and control rats from 1 to 25 days of age. Prenatal stress, Fostering and Sex did not affect the steady state levels of these substances in the central nervous system.

Table 31

Endogenous Levels of 5-HT, 5-HIAA, and NE in the Brainstem Tissue of Fostered and Non-Fostered PMS and Control Offspring. Values Listed are Means for 12 Animals

Age (Days)	Non-Fostered			Fostered		
	1	12	25	1	12	25
5-HT ($\mu\text{g/g}$)						
EPS	.169	.351	.483	.158	.303	.507
LPS	.178	.332	.430	.189	.369	.518
C	.198	.327	.428	.197	.332	.443
5-HIAA ($\mu\text{g/g}$)						
EPS	.161	.284	.477	.185	.324	.447
LPS	.192	.385	.514	.146	.423	.638
C	.203	.341	.472	.198	.353	.510
NE ($\mu\text{g/g}$)						
EPS	.099	.175	.286	.098	.256	.328
LPS	.079	.200	.286	.087	.196	.310
C	.104	.218	.273	.098	.220	.306

DISCUSSION

The results of this experiment indicate that neonatal activity was affected by PMS as in Exp. I. Day 1 activity levels of EPS and LPS pups were significantly elevated above control levels. Over the first 10 days, stressed animals were more active than controls. During the third postpartum week, they were less active than controls. The ontogeny of locomotor activity was accelerated by PMS. Peak activity occurred in the stress groups around 10 days of age. In control pups, maximum activity was not observed until the end of the third week of life.

It should be noted that the direction of the effect on SMA for this experiment was not consistent with that of Exp. I. As stated earlier, it is possible that the changes in neonatal activity observed in the previous experiment reflected the pups' reactivity to the external stimulation of the testing procedure and that what was being measured in the present experiment was the ontogeny of locomotor activity. While this might serve to explain the differences in total activity and the age at which peak activity levels occurred between the groups in the two experiments, it does not satisfactorily explain why the day 1 activity of the PMS pups was lower than that of controls in Exp. I, but increased in Exp. II.

It is possible that this discrepancy in day 1 activity scores reflected the difference in the amount of stress to which the pregnant females in the two experiments were exposed. In Exp. I, females were

exposed to stress either early in the first trimester (vaginal smearing), or to both first and second trimester stress (handling or electric foot shock). Pregnant females in Exp. II were stressed either throughout the first or second trimesters.

If it is assumed that the age-related changes in locomotor activity reflect the ontogeny of behavioral arousal and parallel the development of noradrenergic hindbrain mechanisms and rostral serotonergic systems as has been suggested (Campbell et al., 1969, 1972, 1973), then these data could be interpreted in terms of alterations in brain development as a function of PMS. The precocial development of locomotor activity should therefore reflect a similar change in brain chemistry. However, the postnatal development of endogenous levels of serotonin, norepinephrine, and 5-hydroxyindoleacetic acid in the central nervous system was not altered by prenatal stress. Both stress and control groups exhibited the expected increase in steady-state levels of the monoamines as a function of age (Himwich, 1962; Louizo, 1970, 1972).

Prenatal environment stress has been shown to produce alterations in brain amine levels in the adult offspring (Plaut, Graham, and Leiner, 1972). The hypothesis that these changes reflect alterations in the development of these systems is not supported by the results of the present experiment. Differences in endogenous levels were not apparent between PMS and control offspring at either 1 day of age, before possible existing differences could be reduced or eliminated by interaction with foster mothers; or at 12 days postpartum, an age when bidirectional sex differences have been reported (Guilian et al.,

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1973); or at Day 25, when a number of biochemical processes in brain tissue approach the level of those in the mature animal and the CNS can be considered functionally mature (Himwich, 1962). There is some suggestion that the effects of PMS on brain chemistry are triggered by weaning (Petropoulos, Lau and Liao, 1972) and may therefore only be apparent in the older animal and not during very early life.

Sex differences in NE and 5-HT were also not apparent at 1, 12, and 25 days of age. This is in contrast to the transient increase in 5-HT levels in the female rat brain on postnatal days 10, 12, and 14 reported by Guillian et al. (1973) but are in general agreement with Hardin (1973a, b) for the ages used.

That changes in the ontogeny of locomotor arousal were not reflected in biochemical maturation may also be due to the ages at which biochemical determinations were made. These did not coincide with the ages at which differences in locomotor activity were found between stress and control groups. Alternatively, NE, 5-HT, and 5-HIAA levels may essentially be uncorrelated with behavioral effects.

The sex of the offspring was not found to interact with the effects of PMS on locomotor activity. Sex differences in response to PMS have been reported but primarily for adult offspring, 40 or 90 days of age (Thompson and Quinby, 1964; Weir and DeFries, 1964; Ader and Plaut, 1968; Corson et al., 1970). This would suggest that the sexually dimorphic effects of PMS are dependent upon the appearance of circulating levels of gonadal hormones. The fact that the transient increase in ovarian activity and estrogen production in the female rat pup

between 10 and 14 days of age did not induce sex-related changes in activity at this age would further suggest that there is a critical level of gonadal functioning that must be reached before at least some sexually dimorphic behaviors become apparent. In the rat, after a latent period, estrogen and androgen production is resumed at approximately 35-42 days postpartum, an age which corresponds to reports of behavioral sex differences.

The fostering of PMS pups to non-stressed mothers accentuated the differences in locomotor ontogeny between the EPS and LPS groups and exaggerated differences between both PMS groups and controls. This would suggest that when stressed pups are raised by stressed mothers, any difference in these offspring that are apparent at birth as a function of the gestational period during which the mother is stressed, may be ameliorated by alterations in maternal behavioral and physiological processes that persist after the cessation of the stress. Alternatively fostering may have exaggerated already existing differences between EPS and LPS pups by producing differential maternal treatment. There is evidence showing that mouse parents treat fostered offspring of one type differently from those of another type (Ressler, 1962). Clearly, no such differential treatment can occur unless the litters already differ as a result of prenatal treatment. That the differences between control and stress groups appeared in both fostered and non-fostered stressed pups indicated that this effect originated in utero.

Although PMS seemed here to accelerate the ontogeny of locomotor activity in the developing rat, this change was not reflected in

a general alteration in growth and development. Differences in body weight between stressed and control groups were minimal. Moreover, the appearance of physical features and reflexive behavior was not altered in the prenatal stress group. This would suggest that the CNS structures mediating locomotor activity and those responsible for the maturation of reflexive behavior are differentially sensitive to prenatal stress with the former being more affected.

Spontaneous Alternation

The fact that control groups did not show the expected pattern of development of SA makes these data difficult to interpret. The variation in the developmental pattern in SA in the combined control group occurred primarily after 27 days of age, when alternation behavior dropped again to chance levels. From 17 to 27 days, the sequence was identical to that described by Douglas et al., (1973).

There are several suggestions that the normative data from this study and that of Douglas might not be comparable. The subjects in the latter experiment were from "mothers of heterogenous genetic background" while offspring in the present study were from an inbred Wistar strain. Henderson (1971) has pointed out that hybrid mice show higher rates of alternation than inbred mice. Of six inbred strains tested, only three showed consistent alternation as adults. Data from male Wistar rats (Eggar et al., 1973) indicate however that the adult of this strain does show consistently high rates of alternation, but, in 30-day old Wistars, alternation rates were lower than those observed by Douglas (i.e., 72% and 80-85% respectively). In addition, a recent

study by Blouin (unpublished data) indicates that the pattern of increased alternation rates followed by a decrease to chance levels is the normal developmental sequence for Wistar strain rats in a repeated measures design.

Prenatal stress retarded the age at which alternation behavior first appeared. Control groups showed reliable levels of alternation at 27 days of age. Pooled alternation rates for the four stress groups were not significantly above chance levels until 37 days of age (see Table 33). One possible explanation for these data might be that the lower rates of SA seen in the stress groups reflect their lower overall activity during the third postpartum week. However, the number of animals that failed to run the T-maze on any given day was negligible in both the stress and control groups. The low alternation rates observed in the EPS and LPS pups resulted from their choosing the same goal arm on consecutive trials. Moreover, at 25 and 30 days of age, activity levels in the stress and control groups were not significantly different.

That alternation rates reached adult levels later in the stress than in the control group is not consistent with the notion that the development of locomotor arousal was accelerated in PMS rats. While it would therefore seem logical to interpret the effects of prenatal stress on behavioral development in terms of the dependent variable selected rather than a generalized acceleration or retardation in behavioral development, there is a possible, though highly speculative, reconciliation of these data.

Table 33

Percentage of Alternation Behavior. Data from the Stress Groups has been Pooled Across Stress. Values for Fostered and Non-Fostered Groups have been Collapsed.

<u>Age</u>	<u>Stress Groups</u>	<u>Control Groups</u>
17	48.7	39.0
22	57.5	54.0
27	52.0	62.5
32	58.2	50.0
37	66.0	58.5

Perhaps the accelerated development of locomotor arousal observed in the stressed pups reflects precocial maturation of the CNS. Nicholson and Altman (1972) have shown that accelerated brain development, produced by the administration of thyroid hormone to the neonatal rat, induced a premature termination of cell proliferation and an early initiation of cell differentiation in the cerebral and cerebellar cortices. This resulted in a decrease in brain weight and total cell number in the adult animal. Behaviorally, there was an advance in certain aspects of somatic maturation and reflex development in the neonate. However, the acquisition of several behaviors in older animals was retarded (Eayrs and Levine, 1962).

Accordingly, PMS may accelerate the maturation of the CNS. The immediate effect of this precocial brain maturation is to enhance early behavioral development (i.e. locomotor arousal). The retarded development of later occurring behavior (i.e. spontaneous alternation) might reflect the subsequent alteration in brain weight and cell number. This type of interpretation could also account for the inferior performance of prenatal stress rats in the open field and on several learning tasks that has previously been reported.

EXPERIMENT III

The development of locomotor arousal, spontaneous alternation and reflexive behavior have been linked with the functional maturation of cortical and sub-cortical structures (Eayrs, 1959; Campbell et al., 1969; Altman et al., 1972; Douglas et al., 1972). The development of these structures has been discussed in terms of the functional integrity of the serotonergic, cholinergic and noradrenergic systems (Carlton, 1963, 1968; Fibiger and Campbell, 1971; Moorcroft et al., 1971; Campbell and Mabry, 1972; Egger et al., 1973). Earlier, I hypothesized that changes in the ontogeny of these behaviors as a function of prenatal stress should be reflected in alterations in brain chemistry. However, no differences in endogenous levels of norepinephrine, serotonin, or 5-hydroxyindoleacetic acid were found between control and stressed rats. Either endogenous levels of these amines are not necessarily correlated with behavioral changes or, alternatively, alterations in behavior may be functionally related to alterations in turnover rates of these amines. Eayrs (1959) has pointed out that although the maturation of the nervous system involves quantitative changes in particular constituents which show a well-defined maturational curve, the steady state concentrations of these constituents may not be a sensitive marker for determining changes in tissue maturation.

Experiment III was designed to test whether the behavioral parameters of the preceding experiments were in fact related to central nervous system maturation. This was investigated by examining behavioral ontogeny in rats whose brain development was altered by

neonatal thyroidectomy using ^{131}I , radioactive iodine³.

The effects of thyroid deficiency on brain development are well documented. Hypothyroidism produced a decrease in adult cerebral and cerebellar weights. The deficit in the cerebral cortex results from decreased cell size with a concomitant increase in packing density. In the cerebellum, there is a transient decrease in cell number, presumably due to a reduced rate of cell proliferation in the external granular layer. There is also a permanent reduction in cell size and less differentiation of cells (Eayre and Taylor, 1951; Eayrs and Horn, 1955; Nicholson and Altman, 1972). The reduction in the number of axodendritic synapses in the cerebral cortex is thought to be a consequence of delayed dendritic development with a subsequent decrease in dendritic branching (Eayrs, 1955; Balazs, Kovacs, Teichbraber, Cocks and Eayrs, 1968; Nicholson and Altman, 1972). These anatomical changes have been related to alterations in patterns of energy metabolism that include decreased synthesis of nucleic acid and proteins (Krawiec, Gracia, Argiz, Gomez and Pasquini, 1969), lipids and myelin (Geel and Timeras, 1967) as well as altered enzyme activity in the cerebral and cerebellar cortices (Hamburgh and Flexner, 1957).

Neonatal thyroid deficiency alters several features of brain development which occur at relatively discrete age periods in controls, including cytoarchitectural development of the cerebellum. Under normal

3. Thyroidectomy usually refers to surgical removal of the thyroid gland. Throughout Exp. III and subsequent discussion, thyroidectomy will refer to a hypofunction of the thyroid gland induced by a pharmacological manipulation. It should be noted that ^{131}I radiothyroidectomy may induce glandular and brain alterations different from those caused by surgical thyroidectomy.

conditions, the external granular layer disappears by 21 days of age. If rats are thyroidectomized, this layer persists until 28 days postpartum. However, the development of this structure is not suppressed. If the hypothyroid state is maintained indefinitely, the external layer eventually disappears, but the dendrites of the Purkinje cells in the molecular layer never differentiate to their full extent (Hamburgh, 1966; Hamburgh, Lynn, and Weiss, 1964). In the cerebral cortex, the rate of growth of the cell bodies is diminished resulting in smaller, more tightly packed cells (Balazs *et al.*, 1968). These observations suggest that thyroid hormone plays a key role in the process of cell differentiation (Nicholson and Altman, 1972) and that it is necessary for the proper timing of the transition from cell proliferation to cell migration (Hamburgh, Mendoza, Burkart, and Weil, 1971).

In addition to disrupting structural development, neonatal thyroidectomy alters the ontogenetic changes in biochemical substances in the CNS. Experimental cretinism selectively attenuates the normal increase in succinic dehydrogenase and cholinesterase activity (Hamburgh and Flexner, 1957). This condition also interferes with the normal developmental increase in both the activity of tyrosine hydroxylase and brain norepinephrine levels (Rastogi and Singhal, 1974a) as well as acetylcholine and dopamine metabolism (Ghosh, Merali, Hrdina, and Singhal, 1973). The difference in enzyme activity between normal and thyroidectomized animals first appears at 7 days of age and precedes changes in endogenous levels by 3 weeks. Significant alterations in tryptophan hydroxylase, 5-HT, and 5-HIAA are not evident in thyroxine deficient rats until 28 days of age (Rastogi and Singhal, 1974b).

The effects of neonatal thyroidectomy are not limited to maturational changes in the CNS. A general retardation in growth and development has been reported. The cretinous pup cannot be readily distinguished by its physical appearance from normal litter mates for the first 12 days of life; thereafter marked differences are evident. Ontogenetic changes in the breakdown of epithelial tissue, i.e. eyelid dysfunction and elevation of the auditory external meatus, are retarded. The snout remains foreshortened and sparse infantile hair stands out from the body. Skeletal age is also retarded (Eayrs and Taylor, 1951). Body weight is significantly reduced and the difference becomes more pronounced as the rat matures. These observed alterations in anatomical and biochemical maturation after neonatal thyroidectomy suggest that in early postnatal life, thyroxine may not produce its usual calorogenic effects but may exercise a fundamental role in CNS development. The peripheral metabolic action may appear at a later ontogenetic stage (Eayrs, 1959).

Support for the idea that thyroxine may have a dual function during development comes from reports that there is a critical period during neonatal development prior to which radiothyroidectomy produces structural changes in the brain. If treatment is delayed, these changes are not evidenced. This "critical period" appears to exist between 10 and 14 days postpartum (Eayrs, 1961). These ages also coincide with the time during which replacement therapy with thyroxine (T_4) or L-triiodothyronine (T_3) is effective in reversing the maturational effects of cretinism. Replacement therapy corrects the decrease in

cerebral and cerebellar weight and reverses the alterations in nucleic acid content if it is initiated on or before day 10. When it is delayed until 15 days of age, there is no ameliorating effect (Eayrs, 1958; Ramirez de Guglielmo and Gomez, 1966; Rastogi and Singhal, 1974a, b).

The maturational and metabolic changes induced by neonatal thyroidectomy have been related to behavioral disturbance seen in the adult and neonate. When thyroid deficiency is induced in new-born rats, the age at which certain complex reflexes first appear is delayed. Although the development of the startle response, free-fall righting, reflex suspension, and the placing reflex was retarded, all of these reflexes eventually appeared. Since these behaviors depend upon the maturation of sense organs and subcortical neural connections in the brain to integrate the response as a whole, Eayrs and Lishman (1955) suggested that not only was the development of the assembly of neural mechanisms for simple reflex activity retarded by thyroidectomy but that the control of higher centers over basic reflex mechanisms was likewise delayed.

The behavioral effects of thyroid deficiency arising in the mature animal consist of decreased spontaneous activity (Richter, 1933; Hall and Lindsay, 1938; Brody, 1941), severe impairment in the establishment of a conditioned response (Morrison and Cunningham, 1961), inferior performance on problems involving discrimination (Scow, 1946; Thompson and Kenshalo, 1954), and an increase in the number of errors while learning a maze (Carlson, 1941).

The impairment in the performance of neonatally thyroidectomized rats as adults is somewhat similar to those observed in rats thyroidectomized during adult life, but impairment is more severe, and is thought to reflect early maturational impairment (Eayrs and Levine, 1962). In the mature animal, the effects are attributed to unspecified metabolic changes in the CNS or elsewhere in the body (Eayrs and Lishman, 1955).

The effectiveness of replacement therapy is also related to the age at which treatment with thyroid hormone is begun and the task on which the animal is tested. If rats are thyroidectomized at birth and are then treated with T_3 from the 10th day onwards, their performance is normal as adults. If however they are thyroidectomized at birth and are not treated until after the third week postpartum, their performance is still impaired. This reflects the finding that replacement therapy can reverse the metabolic but not the anatomical alterations when initiated after 24 days postpartum. Neonatal but not delayed thyroidectomy impaired performance in the Hebb-Williams test, a learning test which probably is responsive to the integrity of the cerebral cortex (Eayrs and Levine, 1962). This suggests that the deficit observed in animals thyroidectomized during the first 10 days of life is not simply a consequence of the metabolic effects of thyroid deficiency, but rather reflects a disturbance in development.

It is generally accepted that the behavioral, biochemical, and anatomical effects that result from neonatal radiothyroidectomy are produced by a decrease in the circulating levels of thyroxine. However, the selectivity of ^{131}I refers to its exclusive retention in the thyroid

gland and accessory tissue rather than the specificity of an uptake mechanism. Therefore in the neonatal rat with its underdeveloped blood-brain barrier, these changes possibly reflect the direct effect of a radioactive substance on the brain. At present there is no known definitive evidence for or against this hypothesis. However, the fact that replacement therapy with T_3 or T_4 can reverse some of the maturational and metabolic effects of radiothyroidectomy suggests that the thyroid gland and thyroxine are at least involved.

The anatomical and biochemical alterations seen after neonatal thyroidectomy make the hypothyroid rat an ideal preparation in which to assess the relationship between behavioral ontogeny and central nervous system maturation. The fact that in thyroid-deficient rats, there is a marked retardation of the maturation of the cerebral and cerebellar cortices, and the ontogeny of brain chemistry suggests that the development of behavioral arousal and spontaneous alternation should be retarded or distorted in the cretinous rat. That CNS maturation is delayed but not totally suppressed suggests that a given behavior, or an aspect of it, might eventually appear.

Hypothyroidism was produced in the neonatal rat by an injection of ^{131}I . The use of a radioactive substance necessitated that this experiment be conducted at the University of Ottawa, Department of Pharmacology. Housing and maintenance conditions differed from those used in the previous experiments because they had to conform to an established routine.

Subjects

Male and female Sprague-Dawley (Rattus norvegicus) rat pups were used because injection procedures had been standardized for this strain of rat. Twelve litters with lactating females (Bio-Breeding Labs, Ottawa) were delivered to the laboratory within 4-12 hours after birth. They were individually housed in polycarbonate nesting cages (44 x 25 x 30 cm) with ad libitum water and Purina rat chow. All animals were maintained on a natural light-dark cycle. Litters were not culled prior to injection.

Induced Hypothyroidism

One day old pups were thyroidectomized by an intra peritoneal (i.p.) injection of 200 micro-curries of radioactive iodine, ^{131}I , (Atomic Energy Commission, Canada) as described by Goldberg and Chialkoff (1949). The sodium sulphate salt was dissolved in 0.9% sodium chloride, and each pup received 0.05 ml of solution.

Schwark (1970) has histologically demonstrated that this dose of radioiodine produces a rapid, and discrete destruction of the thyroid gland. The parathyroid is unaffected and accessory thyroid tissue is eliminated. Nine litters were thyroidectomized using this procedure and 3 control litters were injected i.p. with 0.05 ml of 0.09% sodium chloride. Thyroidectomy was assessed by a visual inspection of the tracheal area for remnants of the thyroid gland. The physical appearance of the pups also indicated to some degree the extent of destruction of the

thyroid (Eayrs, 1959).

Behavioural Testing

Infant Activity Four males and 4 females from hypothyroid and control litters were tested at 5, 10, 15, 20, 25, 30, 35, and 40 days of age. Testing was not begun until 5 days of age in order to minimize any effects due to the postnatal injection procedure per se. Animals were tested until 40 days of age because biochemical changes have been shown to be pronounced between 15 and 40 days of age (Rastogi and Singhal, 1974a, b). All pups were tested at each age and each group consisted of offspring from at least three litters.

As in the previous experiments activity was recorded during a 30 minute test session. Activity was recorded with a Selective Activity Meter (Model S) and Printing Counter (Columbus Instruments, Columbus, Ohio, U.S.A.). For all testing ages, pups were placed in a 25.5 x 15.7 x 13.5 cm polycarbonate container which rested on the sensing field. The apparatus was housed in a sound attenuated chamber with lighting and temperature conditions similar to those in Exp. 2. After the first testing session, pups were weighed, ear-clipped and returned to the litter. All pups were weaned at 25 days of age after activity testing and housed in polycarbonate nesting cages (46 x 37.6 x 21.7 cm). Litters were housed as a unit, irrespective of sex.

Development of Physical Features and Reflexes Reflex and physical development were scored for each rat immediately after activity testing. The indices used were the same as those in Exp. 1 and 2 (i.e. eyelid

dysjunction, incisor eruption, startle response and free-fall righting reflex.

Spontaneous Alternation Twelve naive thyroidectomized and 12 naive saline injected controls were tested for alternation behaviour at 17, 22, 27, 32, and 35 days of age. An equal number of males and females were tested at each age. The only variation in procedure from Exp. II was that rats were run during their light cycle (i.e. 0800 hrs. - 1800 hrs.) and therefore tested under normal lighting conditions.

Statistical Analyses The analysis of neonatal activity and body weight data was similar to that employed in Exp. II. Spontaneous alternation data were analyzed using the procedure outlined in the previous experiment.

RESULTS

Neonatal Activity

The mean activity levels of 5 - 40 day old hypothyroid and control pups are shown in Fig. 13. An analysis of variance (Table 34) revealed a significant main effect of Age ($F = 14.940$, $df = 1/14$, $p < .01$) and a Treatment x Age interaction ($F = 6.325$, $df = 1/14$, $p < .05$). However, thyroidectomy did not significantly alter total activity levels. The results of a simple main effects (Table 35) supplemented by Newman-Keuls tests indicated that activity levels in the control groups increased between 5 and 10 days of age, peaked at 15 days and then declined between 20 and 40 days of age. The hypothyroid pups exhibited a different pattern. Activity increased between 5 and 10 days of age but then remained unchanged ($p's < .05$).

Body Weight

Fig. 14 shows the body weight data for 5-40 day old control and hypothyroid rats. The results of an analysis of variance and simple main effects (see Tables 36 and 37 respectively) indicated that the saline injected controls were heavier than the cretinous rats ($F = 49.455$, $df = 1/14$, $p < .0001$). This difference in body weight was first significant at 30 days of age. The rate of growth was also slower in the hypothyroid animal. The controls showed a consistent weight gain between 5 and 40 days of age. After an initial weight increase, between day 5 and 15, the body weight of the hypothyroid rats reached a plateau but continued to show a slight, but non-significant, increase, until.

Figure 13 Mean Total Activity Counts for Hypothyroid and Saline-Injected Control Pups at 5-40 Days of Age.

Figure 14 Mean Body Weight in Grams for Control and Thyroidectomized Pups at 5-40 Days of Age.

30 days of age (see Appendix C).

Physical Features and Reflex Development

Table 38 lists the number of thyroid-deficient and control pups exhibiting physical features and reflexes at each age. The appearance of incisors and the age at which the startle response was elicited in 100% of the animals was not altered by neonatal thyroidectomy. However, the maturation of the free-fall right response and eyelid dysjunction occurred significantly later (Table 39) in the thyroidectomized rats ($F = 16.00$, $df = 1/14$, $p < .001$. $F = 11.67$, $df = 1/14$, $p < .004$, respectively).

Spontaneous Alternation

The results of the tests for alternation behaviour are presented in Fig. 15. Control animals showed an increase in the frequency of SA with age. Perseverative behaviour and chance alternation were seen in 17 and 22 day old pups respectively. Significant alternation was first observed in 27 day old rats and was apparent at 32 and 37 days of age.

Spontaneous alternation was reduced by neonatal radiothyroidectomy ($F = 16.84$, $df = 1/22$, $p < .001$; Table 40). Thyroid animals, for the most part, did not alternate above chance levels.

Table 38

Development of Physical Features and Reflexes for Control and Hypothyroid Rats from 5-40 Days of Age. Values Listed are the Number of Animals Exhibiting Each Variable at a Given Age. Maximum=8

	<u>Group</u>	<u>5</u>	<u>10</u>	<u>15</u>	<u>20</u>	<u>25</u>	<u>30</u>	<u>35</u>	<u>40</u>
Incisors	Hypothyroid	0	1	8	8	8	8	8	8
	Control	0	4	8	8	8	8	8	8
Eyes Open	Hypothyroid	0	0	3	8	8	8	8	8
	Control	0	0	8	8	8	8	8	8
FFR	Hypothyroid	0	0	0	0	5	5	6	8
	Control	0	0	0	8	8	8	8	8
Startle	Hypothyroid	0	0	2	8	8	8	8	8
	Control	0	0	5	8	8	8	8	8

Figure 15 Spontaneous Alternation Data for Hypothyroid and Control Pups at 17, 22, 27, 32 and 37 Days of Age. Values Shown are the Percent of Animals Exhibiting Alternation Behavior at Each Age.

DISCUSSION

The results of this experiment indicate that the ontogeny of locomotor activity and SA are affected by alterations in central nervous system maturation. Radiothyroidectomy of the neonatal rat retards the maturation of the brain and was observed here to disrupt the ontogeny of locomotor arousal. Hypothyroid animals did not show the characteristic increase in activity exhibited by the controls between 10 and 15 days of age. Activity levels in the thyroidectomized rats increased between 5 and 10 days of age and then remained unchanged.

The thyroidectomy-induced alterations in ontogeny of behavioral arousal occurred primarily after 10 days of age. Prior to this time, the activity of the control and hypothyroid pups were indistinguishable. The first 10 days of life corresponds to the critical period during which radiothyroidectomy as well as replacement therapy is effective in the rat. If thyroidectomy is delayed until 15 days of age, maturational changes are not observed. Replacement therapy must be initiated between days 10 and 14, the age period at which many of the biochemical effects of hypothyroidism first appear.

The retarded development following neonatal thyroidectomy would appear to be of a general nature. The appearance of innately organized behavior as well as certain physical maturational changes was delayed. In addition thyroidectomy produced marked impairment in growth as measured by body weight.

The ontogeny of SA was also disrupted following neonatal treatment with ¹³¹I. The reduction in alternation behavior may result from the

reduced sensitivity to environmental stimuli (Eayrs and Levine, 1961), especially since Egger (1973) has related the ontogeny of SA to the animals' response to stimulus novelty.

It was suggested earlier that a given behavior whose maturation was delayed by neonatal thyroidectomy might eventually appear. Recent data indicated that the percentage of spontaneous alternation behavior exhibited by 90 day old thyroidectomized rats was not significantly different from that of age-matched controls. Both groups alternated on approximately 70% of the trials (Sobrian, Edson, and Pappas, unpublished observation).

GENERAL SUMMARY AND DISCUSSION

The main results of these experiments can be summarized as follows:

1) Previous experiments have generally reported that PMS decreases the activity of the offspring in the open field (see Archer and Blackman, 1971). This finding was replicated in Exp. I insofar as offspring of handled and non-handled mothers showed three-minute open field ambulation scores that were lower than those of the control groups. That pups of mothers exposed to electric foot shock during pregnancy showed increased open field activity suggests that in addition to such factors as strain of the animal, fostering, duration and timing of stress, which have been previously shown to determine PMS effects (Archer and Blackman, 1971), the degree of stress to which the pregnant animal is subjected can also influence the direction of the prenatal stress effect.

The electric shock condition may have been unduly stressful due to the inescapability and unpredictable nature of this stressor (Weiss, 1972). In human neonates, morbidity scores were highest in infants whose mothers had been exposed to stressful situations that were continuous or liable to erupt at any time or that were incapable of resolution (Stott, 1973). Moreover, four out of five of these children showed a developmental lag on several indices and most of these children exhibited behavioral abnormalities which were suggestive of congenital hyperactivity.

The change in open field ambulation has been most often interpreted as indicative of alterations in offspring emotionality in PMS research. However, I suggested that it might reflect changes in

adaptation to a novel environment between control and stress pups. The adaptation rates of several open field variables were altered in PMS rats in comparison to controls. However, since there are no other data now available with which to compare these results, a replication of these data are necessary before a definitive statement can be made concerning whether PMS retards or accelerates the development of adaptation to a novel environment. In previous experiments, changes in activity have been reported after repeated exposures to the open field, separated by an inter-trial interval of 24 hours (Thompson, 1957; Doyle and Yule, 1959; Hockman, 1961; Thompson et al., 1963; Weir and DeFries, 1964; Thompson and Quinby, 1964). If these data were analyzed on an overall rather than a trial by trial basis, then any differences in adaptation rates would have been overlooked.

Finally, the adaptation rates of ambulation scores for control rats in Exp. I are in general agreement with normative data for rats given a daily exposure to the open field for 10 consecutive days (Bronstein, 1972a, b; Bronstein, Neiman, Wolkoff, and Levine, 1974). Younger (30-31 days old) and older rats (60 and 70 days old) here showed different adaptation rates to repeated open field trials and both age groups showed a decline in squares crossed from minute to minute.

2) Somatic maturation and reflex development were not affected by PMS. Prenatal maternal dietary deficiency (Smart and Dobbing, 1971) or exposure of the pregnant female to rotating magnetic fields (Ossenkopp et al., 1973) retards the appearance of physical features and nutritional deprivation delays the age at which reflexive behavior

matures. The difference between these findings and those from the present experiment probably reflects the manner in which the fetus is affected by these prenatal treatments. Both undernutrition and exposure to magnetic fields during gestation can directly affect the conceptus (Altman, Das and Sudarshan, 1970; Ossenkopp *et al.*, 1973), while handling and electric shock are thought to mediate their effects indirectly through the mother's response to stress (Joffe, 1969).

The development of endogenous levels of several brain amines was not altered by prenatal maternal stress. Both PMS and control pups showed the characteristic increase in 5-HT, 5-HIAA and NE concentrations during the neonatal period. As stated previously, these negative findings might reflect the fact that steady-state levels of these amines are not sensitive to prenatal manipulations or that biochemical determinations were made at inappropriate ages. However, Giulian, McEwen and Pohorecky (1974) have reported that cold stressing pups on the first postnatal day elevated their brain concentrations of 5-HT over that of controls between 12 and 16 days of age. It would appear that prenatal stress does not have the same effect of brain amines as neonatal stress.

3) Although physical and neurochemical maturation were not disrupted by PMS, the ontogeny of SMA was sensitive to the prenatal manipulations employed in Exps. I and II. However, there were several inconsistencies between the two studies. In Exp. I, PMS increased the total activity of the stressed pups, and delayed the age at which peak activity occurred in comparison to controls. In Exp. II, total activity was unchanged but PMS accelerated the ontogeny of SMA. The sharp increase

in SMA and subsequent decline occurred significantly earlier in the PMS groups.

It is highly probable that both experiments were not measuring the development of spontaneous motor activity. Earlier, it was suggested that the age-related changes in Exp. I might reflect locomotor activity that was elicited by the novelty of the testing situation. This was attributed to the use of a between subjects design and a short test session. Although this kind of explanation might serve to explain the differences in total activity and the age at which peak activity levels occurred between the two experiments, it does not satisfactorily explain why day 1 activity of PMS pups was lower than that of controls in Exp. I, but higher than that of controls in Exp. II. Perhaps this bidirectional effect reflects the differences in the amount and duration of stress to which pregnant rats in the two experiments were subjected. In Exp. I, females were exposed to stress either early in the first trimester, or to both first and second trimester stress. Pregnant females in Exp. II were stressed either throughout the first or second trimester. Day 1 scores might be most likely to reflect the variation in prenatal stress conditions because of the pups more recent exposure to the stressed mothers.

Assuming that Exp. II provided a more valid measure of the ontogeny of spontaneous motor activity than Exp. I, it would appear then that PMS accelerates the development of this behavior. This precocial development was seen in both fostered and non-fostered PMS pups and was not a sex-related phenomenon. In agreement with this interpretation is

the report that prenatal handling advances the age at which the adult-like 24-hour rhythms in activity are first observed (Ader and Deithman, 1970). Stressed pups showed an increase in nocturnal over daytime activity at 5 days of age, whereas controls did not display this rhythmicity until 7 days of age.

Campbell et al. (1969, 1972, 1973) have proposed that the development of SMA reflects the maturation of several systems and structures in the CNS. If behavioral measures can serve to index the structural development of the brain, the results of Exp. II suggest that prenatal stress accelerates CNS development.

4) The maturation of spontaneous alternation was also affected by prenatal stress, in that the age at which PMS pups first showed reliable levels of alternation behavior was delayed. This finding is not consistent with the interpretation that PMS accelerates CNS maturation. It is therefore difficult to formulate an overall interpretation of the data in terms of an acceleration or retardation of behavioral development. Rather, any conclusions concerning the effects of PMS on the ontogeny of behavior might depend upon the dependent variable selected. Alternatively, as was argued in the discussion of Exp. II, the accelerated development of locomotor arousal observed in PMS pups might reflect a precocial maturation of the CNS. However, since accelerated early brain development induces alterations in cell proliferation and differentiation (Nicholson and Altman, 1972), the subsequent deficit in brain weight and total cell number might then affect the development of late-maturing behavior, such as spontaneous alternation. The

generally inferior performance of PMS rats as adults lends support to this hypothesis.

Although I posited a relationship between the change in adult behavior and alterations in behavioral development, there was no a priori theoretical link between the developmental parameters and adult behavior. The neonatal behaviors were chosen because they both exhibited a characteristic developmental pattern. Open field behavior is what is most commonly sampled in prenatal stress experiments. The rationale for the suggestion that changes in adult behavior would reflect alterations in behavioral ontogeny comes primarily from the observation that behavioral development proceeds in an orderly fashion and that any disruption in the sequence will produce a disruption in the end product. However, the findings that both the developmental behaviors are affected by PMS as is adult behavior supports the original hypothesis.

5) Exp. III was originally designed as a methodological study to determine whether the maturation of the behavioral variables used in the previous experiments were sensitive to alterations in CNS development. It also provided the first experimental evidence for the putative role of the thyroid gland in the development of behavior above the reflex level. The extensive alterations in brain maturation induced by neonatal injections of ^{131}I disrupted behavioral, reflexive and somatic development. The effects of thyroidectomy were predictable and consistent with reports of retarded brain development in that both the maturation of SMA and SA as well as somatic and reflexive ontogeny were delayed.

The results of this experiment also added some support to the suggestion of Campbell et al. (1972, 1973) that the sharp increase in locomotor activity seen around 15 days of age is dependent, at least in part, upon the development of glandular systems. Hypo-function of the thyroid gland completely eliminated the expected hyperactivity in animals at this age.

6) The contrast between the developmental alterations observed after PMS and radiothyroidectomy is striking. The effects of PMS were limited to disruptions in behavioral ontogeny, while neonatal treatment with ^{131}I altered behavioral, reflexive and somatic maturation. These changes seen in the hypothyroid rat were consistent in that the development of all of the maturational indices were retarded. However, the behavioral changes seen in the offspring of PMS females were not as consistent insofar as the development of locomotor activity was accelerated while the age at which reliable levels of spontaneous alternation appeared was retarded.

Suggestions for Future Research

This thesis was primarily an initial effort to determine whether or not prenatal stress alters behavioral, somatic and biochemical ontogeny. Physical prenatal manipulations were chosen in order to insure the stressfulness of the situation. However, there was some suggestion that psychological parameters might influence the direction of a prenatal stress effect on open field activity. A systematic manipulation of these variables might provide guidelines

for delineating relevant parameters in the human situation since these variables appear to influence the outcome of human pregnancies as well (Stott, 1973).

In addition to SMA and SA, there are several other behaviors that exhibit characteristic patterns of development. These include passive avoidance (Brunner, 1969; Schulenberg, Riccio, and Stikes, 1971), active avoidance (Klein and Spear, 1969; Feigley and Spear, 1970), and the development of 24-hour retention of escape learning (Nagy, 1972; Nagy, Misanin, and Olsen, 1972). Future research might determine the effects of PMS on the ontogeny of these and other learned behaviors and develop a behavioral profile of the PMS rat pup. This, in turn, might suggest whether PMS produces a deficit in certain basic behavioral processes or whether behavioral alterations are indicative of a generalized performance deficit.

Although the behavioral results of Exps. I and II suggested that prenatal stress might alter the development of the CNS, PMS did not produce changes in endogenous amine levels in the brain. The use of more dynamic indices of development in the immature brain such as the activity of various enzyme systems might provide more positive results. The morphological proliferation, migration and differentiation of several CNS structures could be studied with the labeled thymidine technique of Altman et al. (1968). Furthermore, if the alterations in the maturation of SMA parallel changes in the maturation of the CNS components as Campbell et al. (1969, 1972, 1973) suggest, the possibility exists that they might also reflect an alteration in the age at which

PMS pups are sensitive to pharmacological manipulations.

The results of Exp. III showed the relationship between alterations in CNS maturation and behavioral ontogeny. However, the results of this experiment are suggestive rather than definitive indications of the necessity of thyroid functioning for behavioral maturation. Because of the use of only a saline-injected control it is not possible to determine whether the observed changes following neonatal treatment with ^{131}I were the result of a hypo-function of the thyroid gland, the result of the non-specific effect of a radioactive substance on the developing brain, a result of ionic changes following treatment with iodine or a combination of any of these factors. If the results of properly controlled experiments unequivocally point to the role of the thyroid gland in the ontogeny of behavior, the next step might be to determine if replacement therapy with T_3 or T_4 is effective in reversing these alterations, and if the critical periods reported for inducing the biochemical and morphological changes seen in the hypothyroid rat are the same as those for producing behavioral disturbances.

CONCLUSIONS

1) Experiment 1 replicated the generally reported findings of decreased activity of prenatally stressed rats. The degree of stress to which the pregnant animal was subjected also influenced the open field activity of the offspring. Adaptation rates of open field ambulation, rearing, and grooming were altered in PMS pups.

2) Prenatal maternal stress altered the ontogeny of several behaviors. The development of spontaneous locomotor activity was accelerated while the maturation of spontaneous alternation was retarded. The altered development of SMA in the prenatally stressed pups was accentuated by cross-fostering, but not affected by the sex of the offspring.

It is difficult to formulate an interpretation of these data in terms of an overall acceleration or retardation of behavioral development after prenatal stress. However, the accelerated development of locomotor arousal observed in PMS pups might reflect a precocial maturation of the CNS. Since accelerated brain maturation can ultimately reduce cell proliferation and differentiation, the subsequent deficit in brain weight and total cell number might then affect the development of late-maturing behavior.

3) Somatic development, the appearance of physical features, and the maturation of reflexive behavior were not affected by PMS. This indicated that PMS did not produce a generalized alteration in development. Moreover, there were no effects of PMS on the development of endogenous levels of several brain amines. All groups showed the

expected increase in amine levels as a function of age.

4) Experiment III indicated that the behavioral variables used in the previous experiments were sensitive to alterations in CNS development. It also provided the first experimental evidence for the putative role of the thyroid gland in the development of behavior above the reflexive level. Neonatal radiothyroidectomy retarded behavioral, somatic and reflexive development.

5) The effects of neonatal thyroidectomy on behavioral development were more profound and more consistent than those observed after prenatal maternal stress.

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Appendix A
Statistical Analyses of Data

Table 1

Analysis of Variance for the Effects of Prenatal Maternal Stress on 30 Minute Mean Total Activity Counts of the Offspring of the 5 Treatment Groups: Days 1-30

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Stress	4	4282.3×10^2	4.72	$\leq .001$
Age	12	1997.9×10^3	21.68	$\leq .001$
Stress x Age	48	1623.8×10^2	1.79	$\leq .001$
Error	455	90,786.0		

Table 2

Analysis of Variance for Pre-weaning Activity Data for
C-NF and C-F Pups

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Stress	1	152.30	< 1	
Age	11	6441.6×10^2	8.34	$\leq .0001$
Stress x Age	11	130.15×10^4	1.69	
Error	168	77205.0		

Analysis of Variance for Pre-weaning Activity Data from
Offspring of H and ES Mothers

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Stress	1	2172.8×10^2	2.16	
Age	11	1094.1×10^3	10.89	$\leq .0001$
Stress x Age	11	1680.7×10^2	1.69	
Error	168	1005.0×10^2		

Table 3

Analysis of Variance of Pre-weaning Mean Activity Counts
for N-H, H + ES, and C-NF + C-F Groups

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Stress	2	1081.7×10^3	11.69	$\leq .0001$
Age	11	1808.8×10^3	19.55	$\leq .0001$
Stress x Age	22	1658.9×10^2	1.79	$\leq .016$
Error	444	92,508.0		

Table 4

Simple Main Effects for the Significant Stress x Age Interaction
Obtained for Pre-weaning Mean Activity Counts from the N-H, H + ES,
and C-NF + C-F Groups

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Stress	2	1081.7×10^3	11.69	$\leq .0001$
Day 1	2	2402.9×10^2	2.59	
3	2	1129.9×10^2	1.22	
5	2	1697.3×10^2	1.83	
7	2	3460.5×10^2	3.74	$\leq .05$
9	2	1047.3×10^3	11.32	$\leq .01$
11	2	1487.1×10^2	1.61	
13	2	2245.6×10	< 1	
15	2	4244.1×10	< 1	
17	2	7535.0×10	< 1	
19	2	3125.5×10^2	3.38	$\leq .05$
21	2	1193.0×10^2	1.28	
25	2	3524.9×10^2	3.81	$\leq .05$
Age	11	1808.8×10^3	19.55	$\leq .0001$
Stress N-H	11	5857.5×10^2	6.33	$\leq .001$
H+ES	11	1086.0×10^3	11.74	$\leq .001$
C-NF+C-F	11	6441.5×10^2	6.96	$\leq .001$
Error	444	92,508.0		

Table 5

Analyses of Variance of Trend for Mean Activity Scores of Pre-weaning Offspring from N-H, H + ES, and C-NF + C-F Mothers

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
<u>N-H</u>	11	5857.5×10^2	6.33	$\leq .001$
Linear	1	1078.2×10^3	11.65	$\leq .0001$
Quadratic	1	4051.1×10^3	43.79	$\leq .0001$
Residual	9	1459.8×10^2	1.58	
<u>H + ES</u>	11	1086.0×10^3	11.74	$\leq .0001$
Linear	1	5584.6×10^3	60.37	$\leq .0001$
Quadratic	1	5212.4×10^3	56.35	$\leq .0001$
Residual	9	1277.1×10^2	1.38	
<u>C-NF + C-F</u>	11	6441.1×10^2	6.96	$\leq .001$
Linear	1	9753.3×10^2	10.54	$\leq .001$
Quadratic	1	4551.8×10^3	49.21	$\leq .001$
Residual	9	1731.6×10^2	1.87	
Differences in Linear Trend	2	6079.4×10^5	6.57×10^4	$\leq .0001$
Differences in Quadratic Trend	2	1719.2×10^6	1.85×10^6	$\leq .0001$
Error	444	92,508.0		

Table 6

Analysis of Variance for Pre-weaning Body Weight Data from
N-H, H, ES, C-NF, and C-F Pups

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
<u>Stress</u>	4	92.93	3.13	$\leq .015$
<u>Age</u>	11	5070.10	170.81	$\leq .0001$
Stress x A	44	44.27	1.49	$\leq .026$
Error	408	29.682		

Simple Main Effects for Significant Stress x Age Interaction

<u>Stress</u>	Day 1	4	1.10	< 1	
	3	4	8.67	< 1	
	5	4	3.20	< 1	
	7	4	3.59	< 1	
	9	4	13.12	< 1	
	11	4	11.85	< 1	
	13	4	31.75	1.07	
	15	4	9.81	< 1	
	17	4	75.61	2.54	$\leq .05$
	19	4	45.47	1.53	
	21	4	107.50	3.62	$\leq .01$
	25	4	249.89	8.42	$\leq .001$
<u>Age</u>	N-H	11	1167.02	39.32	$\leq .001$
	H	11	787.81	26.54	$\leq .001$
	ES	11	1,225.76	41.30	$\leq .001$
	C-NF	11	1,225.48	41.29	$\leq .001$
	C-F	11	797.18	26.86	$\leq .001$

Table 7

Mean Body Weight Data for 30 and 60 Day Old PMS and Control Offspring

	<u>Day 30</u>		<u>Day 60</u>	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
N-H	71.8	69.5	225.9	161.7
H	63.9	54.4	197.1	168.5
ES	63.3	58.1	209.2	168.8
C-NF	66.3	57.9	216.2	166.7
C-F	69.5	56.9	220.1	153.8

Analyses of Variance of Mean Weight Data for 30 and 60 Day Old PMS and Control Offspring

<u>Day 30</u>	<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
	Stress	4	197.66	1.13	
	Error	34	173.55		
	Sex	1	205.02	1.21	
	Error	35	168.89		
<u>Day 60</u>	Stress	4	213.81	< 1	
	Sex	1	37171.02	78.90	≤ .0001
	Stress x Sex	4	763.26	1.62	
	Error	50	1095.50		

Table 9

Analyses of Variance for the Appearance of Physical Features and Reflex Development in PMS and Control Pups

	<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
<u>Physical Features</u>	Stress	4	0.30	< 1	
	Age	6	118.01	15.83	$\leq .0001$
	Stress x Age	24	0.30	< 1	
	Error	35	7.46		
<u>Reflex Development</u>	Stress	4	0.75	< 1	
	Age	6	103.10	12.66	$\leq .0001$
	Stress x Age	25	1.42	< 1	
	Error	35	8.14		

Table 12

Analyses of Variance for Open Field Data for Four Dependent Variables in
30 Day Old PMS and Control Pups

		df			
	<u>Source</u>	<u>Conventional</u>	<u>Conservative</u>	<u>F</u>	<u>p</u>
<u>Square</u>					
	Stress	4		1.23	
	Sex	1		1.38	
	Stress x Sex	4		1.34	
	Error	50			
	Trials		1	47.11	≤ .001
	Stress x Trials		4	1.94	
	Sex x Trials		1	< 1	
	Stress x Sex x Trials		4	< 1	
	Error	100	25		
<u>Rearing</u>					
	Stress	4		< 1	
	Sex	1		< 1	
	Stress x Sex	4		< 1	
	Error	50			
	Trials		1	39.75	≤ .001
	Stress x Trials		4	1.46	
	Sex x Trials		1	< 1	
	Stress x Sex x Trials		4	< 1	
	Error	100	25		
<u>Grooming</u>					
	Stress	4		2.04	
	Sex	1		< 1	
	Stress x Sex	4		2.37	
	Error	50			
	Trials		1	< 1	
	Stress x Trials		4	1.66	
	Sex x Trials		1	2.19	
	Stress x Sex x Trials		4	< 1	
	Error	100	25		

	<u>Source</u>	<u>df</u>		<u>F</u>	<u>p</u>
		<u>Conventional</u>	<u>Conservative</u>		
<u>Boli</u>					
	Stress	4		1.37	
	Sex	1		< 1	
	Stress x Sex	4		< 1	
	Error	50			
	Minutes		1	8.00	≤ .01
	Stress x Minutes		4	< 1	
	Sex x Minutes		1	5.62	≤ .05
	Stress x Sex x Minutes		4	< 1	
	Error	100	25		

Table 13

Analyses of Variance of 60 Day Old PMS and Control Pups' Open Field Data
for Four Dependent Variables

		df			
	<u>Source</u>	<u>Conventional</u>	<u>Conservative</u>	<u>F</u>	<u>p</u>
<u>Squares</u>					
	Stress	4		1.09	
	Sex	1		2.18	
	Stress x Sex	4		< 1	
	Error	50			
	Trials		1	62.60	≤.001
	Stress x Trials		4	1.25	
	Sex x Trials		1	1.07	
	Stress x Sex x Trials		4	< 1	
	Error		25		
<u>Rearing</u>					
	Stress	4		3.51	
	Sex	1		< 1	
	Stress x Sex	4		< 1	
	Error	50			
	Trials		1	132.80	≤.001
	Stress x Trials		4	1.22	
	Sex x Trials		1	2.03	
	Stress x Sex x Trials		4	1.07	
	Error		25		
<u>Grooming</u>					
	Stress	4		< 1	
	Sex	1		< 1	
	Stress x Sex	4		1.64	
	Error	50			
	Trials		1	5.39	≤.05
	Stress x Trials		4	< 1	
	Sex x Trials		1	2.50	
	Stress x Sex x Trials		4	< 1	
	Error	100	25		

	<u>Source</u>	<u>df</u>		<u>F</u>	<u>p</u>
		<u>Conventional</u>	<u>Conservative</u>		
<u>Boli</u>					
	Stress	4		1.52	
	Sex	1		10.95	$\leq .002$
	Stress x Sex	4		1.02	
	Error	50			
	Minutes		1	7.31	$\leq .05$
	Stress x Minutes		4	< 1	
	Sex x Minutes		1	2.51	
	Stress x Sex x Minutes		4	1.11	
	Error	100	25		

Table 14

Analyses of Variance for Open Field Grooming Data for 60 Day Old Males and Females from PMS and Control Litters

	df			
<u>Source</u>	<u>Conventional</u>	<u>Conservative</u>	<u>F</u>	<u>p</u>
<u>Males</u>				
Stress	4		4.1	
Error	25			
Sessions		1	7.54	≤.05
Stress x Sessions		4	4.1	
Error	50	25		
<u>Females</u>				
Stress	4		4.1	
Error	25			
Sessions		1	2.81	
Stress x Sessions		4	4.1	
Error	50	25		

Table 16

Analyses of Variance for Trial 1 Open Field Data for 30 Day Old PMS and Control Pups

	df				
<u>Source</u>	<u>Conventional</u>	<u>Conservative</u>	<u>MS</u>	<u>F</u>	<u>p</u>
<u>Squares</u>					
Stress	4		347.06	3.18	$\leq .02$
Error	55		109.07		
Minutes		1	290.1	90.25	$\leq .001$
Stress x Minutes		4	39.31	1.22	
Error	110	55	32.14		
<u>Rearing</u>					
Stress	4		88.142	1.30	
Error	55		67.22		
<u>Grooming</u>					
Stress	4		38.88	≤ 1	
Error	55		53.21		

Table 17

Analyses of Variance for Trial 1 Open Field Data for 60 Day Old PMS and Control Pups

	df				
<u>Source</u>	<u>Conventional</u>	<u>Conservative</u>	<u>MS</u>	<u>F</u>	<u>P</u>
<u>Squares</u>					
Stress	4		117.03	1.19	
Error	55		98.34		
Minutes		1	1013.6	24.00	$\leq .001$
Stress x Minutes		4	75.767	1.79	
Error	110	55	42.236		
<u>Rearing</u>					
Stress	4		200.39	4.19	$\leq .005$
Error	55		47.88		
<u>Grooming</u>					
Stress	4		58.94	< 1	
Error	55		59.49		

Table 18

Analysis of Variance for Mean Activity Counts for Fostered and Non-Fostered PMS and Control Pups (Day 1-30)

Source	df		MS	F	p
	Conventional	Conservative			
Sex	1		8597.4	< 1	
Foster	1		1108.1 x 10 ²	1.51	
Stress	2		8718.1 x 10	1.19	
Sex x Foster	1		6722.2	< 1	
Sex x Stress	2		6585.2	< 1	
Foster x Stress	2		1141.9 x 10	< 1	
Sex x Foster x Stress	2		3758.1 x 10	< 1	
Error	16		7315.1 x 10		
Age		1	9372.1 x 10 ²	16.02	≤ .001
Age x Stress		2	4933.0 x 10	13.85	≤ .001
Age x Sex		1	4113.5 x 10	< 1	
Age x Foster		1	8103.2 x 10 ²	< 1	
Age x Sex x Foster		1	4552.2 x 10	< 1	
Age x Sex x Stress		2	3054.9 x 10	< 1	
Age x Stress x Foster		2	2165.6 x 10 ²	3.70	≤ .05
Age x Sex x Foster x Stress		2	3403.9 x 10	< 1	
Error	540	60	5489.9 x 10		

Table 19

Analyses of Variance for Mean Activity Levels for 1-30 Day Old Pups from Fostered and Non-Fostered EPS, LPS, and C Groups

	df				
	<u>Conventional</u>	<u>Conservative</u>	<u>MS</u>	<u>F</u>	<u>p</u>
<u>EPS</u>					
Foster	1		4927.8 x 10	0.58	
Error	22		8425.1 x 10		
Age		1	4338.6 x 10 ²	6.40	≤ .05
Age x Foster		1	1448.4 x 10 ²	2.14	
Error	198	22	6756.7 x 10		
<u>LPS</u>					
Foster	1		7895.2 x 10	.99	
Error	22		7966.2 x 10		
Age		1	1007.5 x 10 ³	14.85	≤ .001
Age x Foster		1	1587.8 x 10 ²	2.34	
Error	198	22	6782.3 x 10		
<u>C</u>					
Foster	1		5415.0	.13	
Error	22		4030.1 x 10		
Age		1	1116.6 x 10 ³	32.49	≤ .0010
Age x Foster		1	1706.4 x 10 ²	4.96	≤ .05
Erro	198	22	3436.1 x 10		

Table 20

Analysis of Variance of Day 1 Mean Activity Levels for
Non-Fostered and Fostered Stress and Control Groups
Pooled across Sex

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Stress	1	2.3070×10^5	3.78	$\leq .02$
Foster	2	6183.5×10	1.01	
Stress x Foster	2	1463.8×10	< 1	
Error	66	6105.2×10		

Analysis of Variance of Mean Activity Scores for 30 Day
Old Offspring of Stress and Control Mothers

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Stress	2	7229.8×10	2.59	
Foster	1	1534.2×10	< 1	
Stress x Foster	2	3422.5×10	1.23	
Error	66	2787.8×10		

Table 21

Analyses of Variance for 1-30 Day Old Mean Activity Counts for Non-Fostered and Fostered Groups Respectively

Non-Fostered

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Stress	2		7271.5×10	1.39	
Error	23		5205.7×10		
Age		1	4595.1×10^2	6.98	$\leq .05$
Age x Stress		2	2212.3×10^2	3.36	$\leq .05$
Error	297	33	6584.4×10		

Fostered

Stress	2		2588.5×10	< 1	
Error	33		8408.6×10		
Age		1	5188.4×10^2	10.96	$\leq .001$
Age x Stress		2	8237.4×10^2	17.41	$\leq .001$
Error	297	33	4732.5×10		

Table 22

Simple Main Effects for Significant Stress x Age Interaction for Mean Activity Scores from Non-Fostered Stress and Control Offspring (Day 1-30)

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
<u>Stress</u> Day 1	2	1483.7 x 10 ²	2.19	
3	2	4929.7 x 10	< 1	
6	2	3330.4 x 10 ²	5.09	≤ .01
9	2	2739.1 x 10 ²	4.18	≤ .05
12	2	2533.3 x 10 ²	3.87	≤ .05
15	2	3078.0 x 10	< 1	
18	2	4590.4 x 10 ²	7.01	≤ .01
21	2	2807.3 x 10 ²	4.29	≤ .05
25	2	4513.5 x 10	< 1	
30	2	2830.8 x 10	< 1	
<u>Age</u>				
Stress EPS	9	1636.6 x 10 ²	2.50	≤ .01
LPS	9	3278.1 x 10 ²	5.01	≤ .001
C	9	3745.3 x 10 ²	5.72	≥ .001
Stress x Age	18	2212.3 x 10 ²	3.36	≤ .001
Error	297	6578.8 x 10		

Table 23

Trend Analysis of Mean Activity Scores for Non-Fostered
C, EPS, and LPS Pups (Day 1-25)

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
<u>EPS</u>	8	1703.9×10^2	2.59	$\leq .05$
Linear	1	2902.4×10^2	4.40	$\leq .05$
Quadratic	1	6510.5×10^2	9.88	$\leq .01$
Residual	6	7097.7×10	1.07	
 <u>LPS</u>	 8	 2923.3×10^2	 4.44	 $\leq .05$
Linear	1	5842.5×10^2	8.87	$\leq .01$
Quadratic	1	1341.9×10^3	20.38	$\leq .01$
Residual	6	6874.4×10	1.044	
 <u>C</u>	 8	 2839.1×10^2	 4.31	 $\leq .05$
Linear	1	1368.1×10^3	20.77	$\leq .01$
Quadratic	1	3922.7×10^2	5.95	$\leq .05$
Residual	6	8514.0×10	1.29	
 Differences in Linear Trend	 2	 1099.2×10^3	 16.69	 $\leq .01$
Differences in Quadratic Trend	2	7322.5×10	1.11	
Within Cells	264	6584.4×10		

Table 24

Simple Main Effect of the Significant Age x Stress Interaction for Mean Activity Counts from the Fostered Groups

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
<u>Stress</u> Day 1	2	9602.3 x 10	2.03	
3	2	8831.8 x 10 ²	18.66	≤ .01
6	2	5647.3 x 10 ²	11.93	≤ .01
9	2	3123.9 x 10 ²	6.60	≤ .05
12	2	7737.3 x 10 ²	16.35	≤ .01
15	2	7908.8 x 10 ²	16.71	≤ .01
18	2	9385.8 x 10 ²	19.83	≤ .001
21	2	2906.1 x 10 ⁴	614.08	≤ .0001
25	2	9565.6 x 10	2.02	
30	2	7821.3 x 10	1.65	
<u>Age</u>				
Stress EPS	9	4150.4 x 10 ²	8.77	≤ .001
LPS	9	8385.1 x 10 ²	17.71	≤ .001
C	9	5807.1 x 10 ²	12.27	≤ .001
Age x Stress	18	8237.4 x 10 ²	17.41	≤ .001
Error	297	4732.4 x 10		

Table 25

Trend Analysis of Mean Activity Scores for C, EPS and LPS Fostered Groups (Days 1-25)

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
<u>EPS</u>	8	4507.2×10^2	9.52	$\leq .01$
Linear	1	2143.3×10^3	45.29	$\leq .001$
Quadratic	1	1027.4×10^3	21.71	$\leq .001$
Residual	6	7249.3×10	1.53	
<u>LPS</u>	8	9162.4×10^2	19.36	$\leq .01$
Linear	1	2795.8×10^3	59.08	$\leq .001$
Quadratic	1	1590.4×10^3	33.61	$\leq .001$
Residual	6	4905.2×10^2	10.36	$\leq .01$
<u>C</u>	8	5517.0×10^2	11.66	$\leq .01$
Linear	1	2676.4×10^3	56.55	$\leq .001$
Quadratic	1	2121.9×10^2	4.48	$\leq .05$
Residual	6	2541.6×10^2	5.37	$\leq .01$
Differences in Linear Trend	2	3432.7×10^3	72.53	
Differences in Quadratic Trend	2	1682.5×10^2	3.55	
Error	264	4732.4×10		

Table 26

Analysis of Variance for Mean Weight Data for Fostered and Non-Fostered Stress and Control Offspring from 1-30 Days of Age

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Sex	1		78.08	2.00	
Foster	1		66.67	1.70	
Stress	2		140.28	3.59	$\leq .03$
Sex x Foster	1		20.97	< 1	
Sex x Stress	2		4.12	< 1	
Foster x Stress	2		113.31	2.90	
Sex x Foster x Stress	2		7.34	< 1	
Error	60		38.99		
Age		1	34334.95	3738.26	$\leq .0001$
Age x Sex		1	10.16	1.11	
Age x Foster		1	12.81	1.39	
Age x Stress		2	33.27	3.62	$\leq .05$
Age x Sex x Foster		1	0.82	< 1	
Age x Sex x Stress		2	1.91	< 1	
Age x Foster x Stress		2	8.64	< 1	
Age x Sex x Foster x Stress		2	4.66	< 1	
Error	540	60	9.18		

Table 27

Simple Main Effects for Significant Stress x Age Interaction
for Body Weight Data from 1-30 Day Old Offspring of Stress
and Control Mothers

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
<u>Stress</u> Day 1	2	3.64		
3	2	18.24	2.02	
6	2	30.03	3.27	$\leq .05$
9	2	21.23	2.31	
12	2	12.00	1.30	
15	2	26.52	2.88	
18	2	18.65	2.03	
21	2	96.84	10.54	$\leq .01$
25	2	19.44	2.11	
30	2	189.22	20.60	$\leq .001$
<u>Age</u> ES	9	6282.02	683.94	$\leq .0001$
LS	9	1666.1 x 10	1818.98	$\leq .0001$
Control	9	1174.8 x 10	1279.08	$\leq .0001$
Age x Stress	18	33.27	3.62	$\leq .0001$
Error	540	9.18		

Table 29

Analysis of Variance of Physical Features Data for C, EPS, and LPS Fostered and Non-Fostered Pups (Days 9-21)

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Stress	2		.2097	2.05	
Foster	1		.0500	< 1	
Error	66		.1022		
Age		1	24.6080	239.99	≤.001
Stress x Age		2	.1159	1.13	
Foster x Age		1	.0326	< 1	
Stress x Foster x Age		2	.0284	< 1	
Error	264	66	.1023		

Analysis of Variance of Number of Pups in EPS, LPS, and C Groups Exhibiting Reflexes (Days 9-18)

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Stress	2		.4288	1.53	
Foster	1		.1736	< 1	
Error	66				
Age		1	23.4281	83.70	≤.001
Stress x Age		2	.6348	2.27	
Foster x Age		1	.2338	< 1	
Stress x Foster x Age		2	.3362	1.20	
Error	198	66	.2799		

Table 30

Analysis of Variance of Spontaneous Alternation Data for Offspring
of EPS, LPS, and C Mothers 17, 22, 27, 32 and 37 Days of Age

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Foster	1		.178	.319	
Stress	2		.803	1.438	
Foster x Stress	2		.086	.154	
Error	66				
Age		1	1.101	2.138	
Age x Foster		1	.171	.332	
Age x Stress		2	.258	.500	
Age x Foster x Stress		2	1.006	1.953	
Error	264	66			

Table 32

Multivariate Analysis of Variance of the Endogenous Levels of Serotonin (5-HT), 5-Hydroxyindoleacetic Acid (5-HIAA), and Norepinephrine (NE) in 1, 12, and 25 Day Old Offspring in Stress and Control Fostered and Non-Fostered Groups

<u>Source</u>	<u>df Hypothesis</u>	<u>df Error</u>	<u>DV</u>	<u>MS</u>	<u>F</u>	<u>λ</u>	<u>p</u>
Stress	2	180	3			.9477	$\leq .001$
Age	2	180	3			.3383	$\leq .001$
5-HT	2	180		1.48	112.87		$\leq .001$
NE	2	180		.75	102.09		$\leq .001$
5-HIAA	2	180		1.93	75.19		$\leq .001$
Foster	1	180	3			.9803	
Sex	1	180	3			.9888	
Stress x Age	4	180	3			.9392	
Stress x Foster	2	180	3			.9692	
Stress x Sex	2	180	3			.9723	
Age x Foster	2	180	3			.9807	
Age x Sex	2	180	3			.9634	
Foster x Sex	1	180	3			.9986	
Stress x Age x Foster	4	180	3			.9550	
Stress x Age x Sex	4	180	3			.9430	
Stress x Foster x Sex	2	180	3			.9898	
Age x Foster x Sex	2	180	3			.9767	
Stress x Age x Foster x Sex	4	180	3			.9415	

Table 34

Analysis of Variance for Mean Activity Counts for Control and Hypothyroid Rats from 5-40 Days of Age

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Treatment	1		7182.0×10	< 1	
Error	14		1030.2×10^2		
Age		1	4808.9×10^2	$14.94 \leq .01$	
Treatment x Age		1	2035.7×10^2	$6.32 \leq .05$	
Error	98	14			

Table 35

Simple Main Effects for the Significant Treatment x Age Interaction for Mean Activity Levels of 5-40 Day Old Control and Hypothyroid Animals

<u>Source</u>		<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Treatment					
✓ Day	5	1	25.00	<1	
	10	1	1526.8 x 10 ²	1.48	
	15	1	1238.2 x 10 ³	12.02	≤ .01
	20	1	2433.6 x 10	<1	
	25	1	3562.6 x 10	<1	
	30	1	1670.5 x 10	<1	
	35	1	921.12	<1	
	40	1	2907.0 x 10	<1	
Error		14	1030.2 x 10 ²		
Days	Hypothyroid	7	1677.5 x 10 ²	5.21	≤ .01
	Control	7	5138.5 x 10 ²	15.96	≤ .001
Treatment x Age		7	2035.7 x 10 ²	6.32	≤ .05
Error		98	3218.7 x 10		

Table 36

Analysis of Variance of Mean Body Weights Data for Control and Cretinoid Rats from Day 5-40 Postpartum

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservation</u>			
Treatment	1		1861.4 x 10	49.45	$\leq .0001$
Error	14		376.39		
Age		1	1861.5 x 10	256.79	$\leq .001$
Treatment x Age		1	3506.07	48.39	$\leq .001$
Error	98	14	72.49		

Table 37

Simple Main Effects For the Significant Treatment x Age
Interaction for Mean Body Weight

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>p</u>
5	1	2.084	< 1	
10	1	.022	< 1	
15	1	2.424	< 1	
20	1	49.689	< 1	
25	1	1080.765	2.87	
30	1	7030.822	18.68	$\leq .001$
35	1	1464.7 x 10	38.91	$\leq .001$
40	1	2034.9 x 10	54.00	$\leq .001$
Error	14	376.392		
Hypothyroid	7	424.533	5.85	$\leq .01$
Control	7	1910.6 x 10	263.5	$\leq .001$
Error	98	72.49		

Table 39

Analysis Variance of the Appearance of Eyelid Dysjunction for Control and Hypothyroid Rats for 10, 15, and 20 Days of Age

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Treatment	1		.521	11.67	$\leq .004$
Error	14		.045		
Age		1	4.187	93.80	$\leq .0001$
Treatment x Age		1	.521	11.67	$\leq .001$
Error	28	14	.045		

Analysis of Variance of the Development of the Free-Fall Righting Reflex in Thyroidectomized and Control Rats at Days 20-40

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Treatment	1		3.200	16.00	$\leq .001$
Error	14		.200		
Age		1	.544	12.43	$\leq .01$
Treatment x Age		1	.544	12.43	$\leq .01$
Error		14	.044		

Table 40

Analysis of Variance of Spontaneous Alternation Data for Thyroidectomized and Saline Injected Controls at 17, 22, 27, 32, and 37 Days of Age

<u>Source</u>	<u>df</u>		<u>MS</u>	<u>F</u>	<u>p</u>
	<u>Conventional</u>	<u>Conservative</u>			
Treatment	1		6.533	16.84	$\leq .0001$
Error	22		.387		
Age		1	2.318	4.36	$\leq .05$
Treatment x Age		1	1.033	1.94	
Error	88	22	.531		

Appendix B

Biochemical Assay Procedure for
the Fluorometric Determination of
Serotonin, 5-Hydroxyindoleacetic
Acid and Norepinephrine

ASSAY PROCEDURE

Preparation of Tissue

The frozen brainstem tissue was weighed and then homogenized in 3 ml of acidified butanol (0.2 ml concentrated HCl in 200 ml n-butanol). The homogenate was centrifuged at 5000 rpm for 5 minutes. The supernatant fluid (2.5 ml) was removed to a tube containing 5 ml n-heptane and 0.5 ml 0.1 N HCl. This solution was placed in a mechanical shaker for 10 minutes, and then re-centrifuged at 4500 rpm for 5 minutes. The aqueous layer was removed and kept for 5-HT and NE assays. The organic layer (5 ml) was removed to a tube containing 0.6 ml of 0.5 M tris buffer, pH 7. After 10 minutes in a mechanical shaker, this mixture was centrifuged at 4500 rpm for 5 minutes. The aqueous layer was removed and assayed for 5-HIAA.

5-HT Assay The 5-HT solution (0.2 ml) was placed in a tube containing 1.2 ml OPT in 10 N HCL and 0.02 ml of 1% cysteine was added. This solution was mixed, then heated at 60° C for 15 minutes. The tubes were then cooled to room temperature and the fluorescence was read at 360/470 millimicrons. The standard consisted of 1.0 micrograms of 5-HT (free base) in 1 ml of 0.1 N HCL. Blanks contained 0.1 N HCL.

5-HIAA Assay The 5-HIAA solution (0.4 ml) was placed in a tube. The following reagents were added in the order given, shaking between each addition: (a) 0.05 ml of 1% L-cysteine, (b) 1.0 ml concentrated HCL, (c) 0.05 ml OPT in methanol and (d) 0.05 ml sodium periodate solution. The mixture was heated at 60° C for 15 minutes, cooled to room temperature, and fluorescence read at 360/470 millimicrons. The standard was

1.0 micrograms of 5-HIAA (free base) in 1 ml of 0.05 M tris buffer, pH 7.

Blanks consisted of 0.5 M tris buffer, pH 7.

NE Assay The NE solution (0.2 ml) was pipetted into a tube containing 0.6 ml M phosphate buffer, pH 7. The following solutions were added: (a) 0.05 ml iodine, (b) 4 minutes later, 0.5 ml alkaline sulphite and (c) 5 minutes later, 0.2 ml glacial acetic acid. This mixture was left at room temperature for 20 minutes; fluorescence was then read at 380/480 millimicrons. The standard contained 1 microgram of NE (free base) in 1 ml of 0.1 N HCl. The blank consisted of 0.1 N HCl.

Appendix C

Physical Appearance of 60 Day
Old Control and Hypothyroid,
Rats and the Thyroid and Adrenal
Glands of these Animals

Figure 16 Physical Appearance of Two 60 Day Old Rats. The One on the Left was Injected with 200 Micro-Curies of Rado-active Iodine Shortly After Birth. The Rat on the Right is a Saline-Injected Control.

Figure 17 Physical Appearance of the Adrenal and Thyroid Glands of a Hypothyroid and a Control Rat at 60 Days of Age. The Thyroidectomized Animal's Adrenals are the Lower Pair. It's Thyroid Gland is on the Right.

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