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THE BEHAVIORAL DEVELOPMENT OF HYPOTHYROID AND HYPERTHYROID RATS

by

Ingrid Johanson Bennett

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March 6, 1978
date

Dr. Gerald Turkewitz,
Co-Chairman of Examining Committee

3/7/78
date

Max Hamburgh
Dr. Max Hamburgh,
Co-Chairman of Examining Committee

March 13, 1978
date

Florence L. Denmark
Dr. Florence Denmark,
Executive Officer

Dr. Gerald Turkewitz
Dr. Max Hamburgh
Dr. H. Philip Zeigler
Supervisory Committee

Abstract

THE BEHAVIORAL DEVELOPMENT OF HYPOTHYROID AND HYPERTHYROID RATS

by

Ingrid Johanson Bennett

Advisers: Dr. Gerald Turkewitz
Dr. Max Hamburgh

The purpose of the present investigation was to analyze the role of thyroid hormones in behavioral development in rats. Charles River CD strain rats were assigned to one of four treatment groups: Group 1, hypothyroid treatment group - the mothers received a diet of 0.2% propylthiouracil starting on day 15 of pregnancy and the offspring were injected with saline starting at birth; Group 2, hyperthyroid treatment group - the mothers were maintained on a standard diet and the pups received excess thyroxine starting at birth; Group 3 - replacement therapy treatment group - the mothers received propylthiouracil and the pups received thyroxine replacement therapy starting at birth; and Group 4, the control treatment group - the mothers were maintained on a standard diet and the pups were injected with saline. First, to determine whether maternal care could serve as an indirect mediator for thyroid hormone effects on behavior, nesting, nursing, grooming, contact and retrieval were assessed in mothers of hypothyroid, hyperthyroid and control litters, as well as mothers of litters receiving thyroxine replacement therapy. Mothers of hypothyroid litters showed a delay in the decline of nesting, nursing and contact, while the decline of nesting was accelerated in hyperthyroid litters. Retrieval of pups was not affected, but grooming of pups by mothers occurred less frequently in hypothyroid and replacement therapy litters. Maternal behavior shown by mothers of hypothyroid pups was considered adequate and in fact may alleviate some of the effects of perinatal hypothyroidism, making it unlikely that deficits in maternal behavior account for the more severe effects of hypothyroidism. Second, to assess the effects of hypo- and hyperthyroidism on an integrated behavioral response, pups in the four treatment groups were tested for the development of the home orientation response. Soiled shavings were placed in the corner of the home cage which had contained the nest ("the home corner"), and fresh shavings were placed in each of the other three corners. The ability of the pups to initiate and maintain locomotion toward the home corner was assessed between days 4 and 22. Hyperthyroid, control and replacement therapy pups behaved very similarly on the task, showing a peak in the percentage of pups homing between days 12 and 16. Hypothyroid pups showed a delay in the peak percentage until day 20, although the percentage of pups was similar to that found in other treatment groups. It therefore seems that an integrated behavior response can be delayed by hypothyroidism, and still emerge apparently intact at a later age. For the final portion of the investigation, pups were assessed for their responsiveness on an olfactory and a thermal gradient, in an attempt to evaluate the ability of the pups to use these cues in orientation. Hypothyroid pups showed a delay and hyperthyroid pups an acceleration in

the development of orientation along the olfactory gradient. There was no evidence that any of the pups were able to utilize the thermal gradient in directed orientation, but pups in all treatment groups moved less at the warm end of the gradient than at the cool end. This differential responding was strongest in the hypothyroid pups, making it possible that these pups could utilize thermal cues in a kinetic orientation.

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Ms. Pat Dash assisted me in the collection of maternal behavior and pup home orientation data. The apparatuses used to establish the olfactory and thermal gradients were made by my father, John H. Johanson. The thyroxine was generously provided by Smith Kline and French Laboratories, Philadelphia, Pennsylvania.

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Ingrid Johanson Bennett
February, 1978

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I. INTRODUCTION

Thyroid hormones are believed to play an important role in the development of the mammalian nervous system. Severe and apparently permanent effects can be seen in the brain and behavior of both animals and humans who, during critical periods of their development, have either been deprived of their normal supply of thyroid hormones or have been exposed to excessive levels of thyroid hormones. A substantial literature exists which documents the effects of early thyroid hormone manipulation on brain biochemistry and morphology. Indeed, extensive research on the role of the thyroid gland in development indicates that thyroid hormones may be important for the normal timing of cellular events, such as proliferation and differentiation (Hamburgh, 1968; Hamburgh, Mendoza, Burkart and Weil, 1971). By changing the timing of these events, changes may occur in the neural organization, and hence behavior.

Effects on Morphology and Physiology

An insufficiency or excess of thyroid hormone seems to affect many aspects of development. There is a marked effect on the appearance of the young animal, with hypothyroid rat pups appearing relatively immature (Scow and Simpson, 1945) and hyperthyroid pups apparently maturing more quickly than controls (Khamsi and Eayrs, 1966). Insufficiency or excess of hormone in the perinatal period seems to permanently affect the functioning of the hypothalamo-pituitary-thyroid axis (Eayrs and Holmes, 1964; Bakke, Gellert and Lawrence, 1970;

Bakke, Lawrence and Robinson, 1972; Bakke, Lawrence and Wilbur, 1974; Bakke, Lawrence, Bennett and Robinson, 1975), as well as the hypothalamo-pituitary-gonadal axis (Gellert, Bakke and Lawrence, 1971) and the hypothalamo-pituitary-adrenal axis (Meserve and Leathem, 1973, 1974). Numerous studies of early hypothyroidism and hyperthyroidism have demonstrated a marked effect on the metabolic and biochemical maturation of the brain. Those enzymes associated with the mitochondrial and synaptosomal fractions of the cell appear to be most affected (Dunn, 1972), for example, GABA transaminase (Gomez, 1971; Pesetsky, Burkart and Hamburgh, 1973) and glutamic acid decarboxylase (Balázs, Cocks, Eayrs and Kovács, 1971). The metabolism of serotonin and norepinephrine is likewise affected (Rastogi and Singhal, 1974 a, b). Furthermore, Valcana and Timiras (1971) report changes in brain ionic metabolism in hypothyroid rat pups and numerous investigators report that protein synthesis is affected (Balázs, _et al_., 1971; Patel and Balázs, 1971; Jarlstadt and Norstrom, 1972; Dainat, 1974). The morphology of both cortex and cerebellum is severely affected by perinatal hypo- or hyperthyroidism. There are particularly marked effects on the development of the neuropil (Eayrs, 1955; Horn, 1955; Eayrs, 1961, 1964 a; Cragg, 1970; Nicholson and Altman, 1972 a, b), and on neuronal and glial cell number (Nicholson and Altman, 1972 c; Clos and Legrand, 1973). Myelination is accelerated in tissue culture by the addition of thyroid hormone (Hamburgh and Bunge, 1964) and myelination is delayed in hypothyroid rat pups (Clos and

Legrand, 1969, 1970; Faryna de Raveglia, Gomez and Ghittoni, 1972; Rosman, Malone, Helfenstein and Kraft, 1972; Bass and Pelton, 1973). The development of neural excitability is also affected in hypo- and hyperthyroid animals, with hypothyroid rat pups showing a delay in the maturation of a normal EEG pattern and hyperthyroid rats an acceleration in development (Bradley, Eayrs and Schmalbach, 1960; Guérin and Gali, 1974; Salas and Schapiro, 1970; Hatotani and Timiras, 1967).

Effects on Behavior

The behavior of hypo- and hyperthyroid animals has also been examined, in an attempt to discover behavioral correlates of these observed neural deficits. For the most part, such studies have limited themselves to a documentation of the time of emergence of various reflex responses, such as the placing, righting and startle responses, and to the examination of adult learning abilities.

Eayrs and Taylor (1951) report a significant delay in the emergence of the placing reflex and the righting response in young hypothyroid rats. Eayrs and Lishman (1955) report that hypothyroid animals spend significantly less time hanging on a reflex suspension test and also show a significant delay in the emergence of the startle response to an auditory stimulus. Eayrs (1964 b, 1968) and Khamsi and Eayrs (1966) report an acceleration in the appearance of the righting and placing responses in hyperthyroid rats, the degree of acceleration showing a positive relationship with hormone dosage. Hyperthyroid animals also show an acceleration of the appearance of the startle response from 3 to 5 days in rats (Schapiro and Norman, 1967) and 1 to 2 days in mice (Hamburgh and Vicari, 1957). Several studies of the ontogeny of swimming in young hyperthyroid rats have found that the development of swimming ability is also accelerated by approximately 2 days (Schapiro, Salas and Vukovich, 1970; Davenport and Gonzalez, 1973; Anderson and Schanberg, 1975), and Hamburgh and Vicari (1957) report a similar acceleration of swimming behavior in mice. Thus, it appears that behavioral development is accelerated in hyperthyroidism and delayed in hypothyroidism, a finding which agrees with the effects of hyper- and hypothyroidism on biochemical, morphological and neurophysiological maturation.

Studies of juvenile and adult learning abilities in both hypo- and hyperthyroid subjects have attempted to assess the extent to which the behavioral effects observed during early development result in permanently altered behavioral responsiveness. Eayrs (1971) and Eayrs and Levine (1963) report deficiencies in adult performance on a simple T maze and on an active avoidance paradigm in neonatally thyroidectomized rats. However, thyroxine replacement therapy, even in adulthood, restored performance to control levels. In the active avoidance paradigm, the speed of the response is particularly important. An effect of metabolic factors on the latency of the evoked cortical response has been found by Eayrs, Glass and Broadhurst (1962), and it may well be that the deficit observed in the active avoidance paradigm reflects the effects of metabolic factors on response latency.

Eayrs (1961), using a Hebb-Williams closed field test, reports permanent deficits in the performance of neonatally thyroidectomized rat subjects. Furthermore, he found that the number of errors shown could be reduced by delaying the onset of hormone removal. Thyroidectomy after 10 days produced animals which performed as well as control subjects. In addition, replacement therapy in neonatally thyroidectomized rats was successful in restoring performance if started before 10 days of age, although it was unsuccessful if initiated at 24 days. It thus seems as if this deficit reflects permanent effects of hormone removal during a limited period of development. Davenport and Dorcey (1972) have also reported deficits in learning an enclosed maze in hypothyroid rats, but only if hypothyroidism was maintained postnatally. Prenatal treatment alone had no effect on maze performance. Davenport (1970) reports impaired performance on a Y maze, as well as on an automated symmetrical maze described by Davenport, Benson, Hagquist, Rankin and Shelton (1972), in animals treated with tricyanoaminopropene (TCAP), an antithyroid drug which acts by inhibiting accumulation of iodine by the thyroid (Turner, 1971).

An interaction between environmental stimulation and subsequent performance has been reported in rats malnourished in infancy by Levitsky and Barnes (1972). They found that the effects of early malnutrition may be alleviated by environmental enrichment. Similarly, it seems that the deficits in performance in maze learning in hypothyroid rats can be alleviated if at weaning the rats are placed in an enriched environment (Davenport, Gonzelez, Carey, Bishop and Hagquist, 1976). The effects of a stimulating post-weaning environment are reported to persist for more than four months. It seems likely that the enriched environment has stimulated synaptogenesis and the formation of dendritic spines, thus alleviating some of the effects of neonatal hypothyroidism on the development of the synaptic neuropil.

The early acceleration of behavioral development shown by hyperthyroid subjects suggests that performance in adulthood might also be better than that of controls. Indeed, Schapiro and Norman (1967) and Schapiro (1968) report that hyperthyroid rats acquire a conditioned active avoidance response more rapidly than controls. These findings on the acquisition of the active avoidance response were not confirmed by Davenport and Gonzalez (1973). They failed to find a difference between hyperthyroid animals and controls on either an active or passive avoidance paradigm, although differences in procedure, apparatus and strain of rat may account for the discrepancy. In any case, as it is possible that metabolic factors might be important in influencing response latencies, the speed of the acquisition of the response by hyperthyroid animals might only reflect current metabolic factors, and not a permanent behavioral effect.

In fact, there is good evidence that adult learning performance by hyperthyroid rats may in fact be impaired. Davenport and Gonzalez (1973) and Davenport, Hagquist and Hennies (1975) found that rats injected neonatally with thyroid hormones performed poorly on an automated symmetrical maze. The deficits shown by animals made hyperthyroid with triiodothyronine were reported by Davenport et al. (1975) to be very similar to those found after neonatal thyroidectomy. It seems then that the early acceleration of behavioral development shown by hyperthyroid animals is followed in adulthood by permanent behavioral deficits, of a magnitude comparable to that found in hypothyroid animals.

Statement of the Problem

The present investigation into the effects of thyroid hormones on behavioral development will address itself to the following issues:

1) One question that has been consistently raised in the literature concerns the problem of whether the various effects of thyroid hormone manipulation on brain and behavior development are indeed direct effects, and various indirect mediators have been proposed, e.g. malnutrition and alterations in pituitary function. One possible indirect mediator that has not been examined is quality of maternal behavior and the question may be raised as to whether some of the behavioral changes observed in young hypothyroid and hyperthyroid rats can be explained by differences in the maternal behavior shown by the mothers of these animals. The first portion of the present investigation will consist of an analysis of the extent to which the maternal behavior of the mothers of hypothyroid and hyperthyroid litters is affected by thyroid hormone manipulation. It is hoped that the findings will

indicate whether maternal factors can serve as indirect mediators of thyroid hormone effects on development.

2) For the most part, investigations of the behavior of young hypothyroid and hyperthyroid animals have been limited to a documentation of the emergence of various reflex responses, while the effects of thyroid hormone deprivation or excess on the development of an integrated behavioral response has not been examined. The second portion of the investigation will thus consist of an analysis of the ability of young hypo- and hyperthyroid rats to display homing behavior, which requires an integration of sensory and motor systems into a coordinated behavioral pattern. By comparing the pattern of responding shown by hypo- and hyperthyroid rat pups with the patterns shown by pups subjected to other types of stunting (e.g. malnutrition) it may be possible to differentiate those aspects of homing which are affected specifically by the thyroid hormone manipulation from those affected by more general features of the experimental treatment (e.g. reduction in body weight).

3) The literature to date does not deal with the development of any of the sensory systems in young hypo- or hyperthyroid animals. Hence, the final portion of the investigation will consist of an analysis of the pups' abilities to utilize olfactory and thermal cues in orientation. It is hoped that an analysis of the sensory capabilities of hypo- and hyperthyroid rats will provide some information about the basis of the behavioral deficits shown. Furthermore, if a specific sensory deficit is found, future research can determine

whether some of the reported effects of hypo- or hyperthyroidism are in fact secondary effects of the primary deficit in sensory function.

It is hoped that the results from this set of studies will clarify some of the ways in which thyroid hormone insuffiency or excess can affect neural and behavioral development. By examining the relationship between structure and function, we may better understand the significance of the induced changes in biochemistry and morphology for behavior development.

II. MATERNAL BEHAVIOR AS AN INDIRECT MEDIATOR OF THYROID EFFECTS

Introduction

A myriad of effects of early hypo- and hyperthyroidism is seen at all levels of organization - biochemical, morphological, neurophysiological and behavioral. The question may then be asked whether the effects of thyroid hormone insufficiency or excess are direct effects on brain development, or whether the effects are mediated indirectly.

Insufficiency of pituitary growth hormone (GH) and malnutrition have been considered two consequences of early thyroid hormone deprivation which may be responsible for the alterations in brain and behavior development observed in hypothyroid animals. However, the deficits observed in animals with an insufficiency of GH or in malnourished animals are sufficiently different from deficits observed in hypothyroid animals to suggest that most of the effects of hypothyroidism are not mediated via changes in pituitary functioning or malnutrition (see, for example, Geel and Timiras, 1971; Horn, 1955; Clos and Legrand, 1969, 1970; Rebière, Bout and Legrand, 1972; Rebière and Legrand, 1972).

One factor which may be important in mediating the effects of early hormone manipulation is the quality or quantity of the maternal behavior shown by the mothers of hypo- and hyperthyroid animals. This problem is particularly serious, since the behavior shown by a mother has been shown to have marked effects on the behavior of her offspring.

Denenberg, Ottinger and Stephens (1962) performed a variety of manipulations on mother rats and measured the behavior of their offspring. They found differences in body weight, emotionality and active avoidance responding, depending on the type of maternal manipulation. The effects of maternal behavior may even carry across generations. Denenberg and Rosenberg (1967) found that the amount of handling received by a female rat in infancy had effects not only on the behavior of her own pups, but also on the behavior of the pups of her female offspring.

The common method of inducing perinatal hypothyroidism by giving the pregnant or lactating female a diet including thiouracil may have the effect of inducing hypothyroidism within the mother herself. While hypothyroidism induced in adult animals has none of the severe effects seen in neonatal hypothyroidism, the possibility remains that maternal hormone deficiency affects the quality of her care of pups.

Studies of the effect of thyroid hormone deprivation in adults on the care of young has demonstrated an enhanced capacity to display parental behavior in thyroidectomized male rats (McQueen-Williams, 1935 a, b). The mother's level of thyroid hormone is likely to be important in the initiation, maintenance and decline of lactation. Mixner and Turner (1942) report a reduced amount of lobule-alveolar growth in the mammary gland of thyroidectomized mouse subjects. Grosvenor and Turner (1959) injected excess thyroxine into lactating rats and found an increase in milk secretion. They suggest that suboptimal levels of thyroxine might produce reduced milk secretion. Perhaps the reduced intramammary pressure in mothers fed thiouracil has an effect on maternal behavior. Moltz, Geller and Levin (1967) report that total mammectomy had no effect on maternal behavior in rats, but Galler and Turkewitz (1977) found effects of partial mammectomy on such behaviors.

Nest building also appears to be affected by the thyroid gland. Adult males and non-lactating females will display a certain amount of nesting, the level of nesting activity depending on ambient temperature. Richter (1941) reports that such nonmaternal nesting can be greatly increased in both males and females by the removal of the thyroid gland. He states that thyroidectomy results in decreased heat production in the adult, and as a result, the rats seek to reduce heat loss by constructing a large nest. It is reasonable to expect that mothers fed thiouracil and hence made hypothyroid might also show increased nesting behavior.

Maternal retrieval may also be affected by the mother's hypothyroidism. It is possible that maternal hypothyroidism would result in increased latencies to retrieve pups, possibly by affecting maternal metabolic state.

Thus it is reasonable to expect that a slight thyroid hormone insufficiency in the female may influence maternal behavior directly. For this reason, it is important to determine the quality of maternal care shown by mothers fed thiouracil.

Maternal behavior can also be affected by stimuli emanating from the young themselves. This problem has been reviewed by Richards (1966) and Meier and Schutzman (1968). They suggest that some of the effects observed in pups handled during early development may actually be mediated via changes in the behavior of the mother and may not reflect direct effects on the pups themselves. Young (1965) addressed this problem experimentally by handling young rat pups and then observing the maternal behavior displayed after handling. He found that mother rats significantly preferred untreated pups, showing a marked tendency to retrieve these pups before treated pups. Barnett and Burn (1967) report that ear-clipping a young mouse increases the quantity of maternal attention received. Treatment of the pups can not only influence maternal behavior, but can also affect other aspects of the mother's behavior, for example, her emotional responsiveness. Hudgens, Chilgren and Palardy (1972) handled pups during the first week postnatally, and then fostered the pups to mothers whose own pups had not been handled. After fostering, pups were not handled and the degree of emotionality shown by the foster mother was determined. The foster mothers of handled pups, whose own litters had not been handled, were considerably less emotional than the foster mothers of unhandled animals, indicating that the effect was mediated via the pup. These differences in maternal behavior may then produce later differences in the behavior of their offspring, as Denenberg _et_ _al_. (1962) demonstrated.

In studies on the effects of hypothyroidism and hyperthyroidism, there is not only an effect on the behavioral maturity of the infant rat, but also on the physical appearance of the animal. It is reasonable to assume that cues from the pups, emanating either from their appearance or behavior, will have effects on the quality and quantity of maternal behavior shown by their mothers.

The possibility that stimulation from the pups may influence maternal behavior was investigated by Rosenblatt (1967, 1969). He permitted females to rear pups until 9 to 14 days postpartum, after which the pups were removed. The females were then tested for a decline in maternal behavior, using 5 to 10 day old test pups. Removal of the young at 14 days resulted in a slowed decline of maternal behavior, suggesting that stimulation from older pups may actively promote a decline in maternal behavior. Nicoll and Meites (1959) found that lactation could be maintained for over two months in rats by replacing litters every ten days with an equal number of 4 day old pups. Thus, one of the factors involved in the cessation of nursing is likely to be the change in stimulation received from the young as they mature. Hormone deficiency or excess in the young may also affect maternal nesting behavior. Hyperthyroid young become active at an earlier age than controls and may contribute to an earlier destruction of the nest, while hypothyroid animals, being relatively lethargic, might permit nest integrity to be maintained for a longer period of time. In addition, hypo- and hyperthyroid animals might be affecting maternal nest building via the relative immaturity or maturity of their appearance or behavior. Since treated pups present a set of stimuli which differs from the normal pup, it is possible that pup hormonal condition may either extend or prematurely terminate maternal retrieval. Beach and Jaynes (1956) found that a variety of sensory cues - visual, haptic and olfactory - serve during the retrieval of pups. It is quite conceivable that the changing stimulus properties of normal young are important in the decline of retrieval behavior during the litter period. The delay or acceleration in the disappearance of these stimuli might thus be expected to alter retrieval behavior.

Thus, one might expect that mothers of hypothyroid pups, being exposed to comparatively immature pups for a longer period of time, would show a delay in the decline of maternal behavior. Similarly, mothers of hyperthyroid pups should show an accelerated decline in maternal responsiveness, since their pups appear to be both behaviorally and physically mature relative to controls.

The maternal behavior of mothers of hypo- and hyperthyroid animals has never been examined, however. Thus, in order to assess the possibility that differences in the behavior of the mothers could account for the observed differences in behavior among the pups, the maternal behavior of mothers of hypothyroid and hyperthyroid infant rats was examined.

Design

Four treatment groups were observed in the present investigation: Group 1, hypothyroid treatment group - the mothers received a diet of 0.2% propylthiouracil starting on day 15 of pregnancy and the offspring were injected with saline starting at birth; Group 2, hyperthyroid treatment group - the mothers were maintained on a standard diet and the pups received excess thyroxine starting at birth; Group 3, replacement therapy treatment group - the mothers received propylthiouracil and the pups received thyroxine replacement therapy starting at birth; and Group 4, the control treatment group - the mothers were maintained on a standard diet and the pups were injected with saline.

There are several reasons for including the replacement therapy treatment in the present study. The level of thyroxine in the mothers of hypothyroid pups may be reduced, since propylthiouracil is first given to the pregnant and lactating female. Therefore, it cannot be determined from a comparison with mothers of normal control litters whether it was the hormonal state of the pup or that of the mother that was responsible for any differences in maternal care received by hypothyroid pups. For this reason, the mothers of one group of litters received propylthiouracil (PTU) while their litters received thyroxine replacement therapy. If the determining factor was the mother's hormonal condition, one would expect no differences in maternal behavior between mothers of pups kept hypothyroid and mothers of pups receiving replacement therapy. On the other hand, if the important factor was the hormonal condition of the pup, there should be a difference in the maternal care shown by these two groups of mothers.

The replacement therapy treatment also served as a control for the possible prenatal effects of thyroid deprivation, as well as a control for any teratogenic effects of PTU not mediated by an insufficiency of thyroxine.

Assessment of maternal behavior in litters in which the pups were hypothyroid and the mothers normal may have answered more directly the question of whether differences in the appearance or behavior of the hypothyroid infant has an effect on maternal behavior. This treatment group was not included in the present study, however, since there are serious technical and theoretical considerations involved in the two techniques used to render pups hypothyroid without treatment of the female. For example, surgical thyroidectomy of a newborn rat involves removal of the parathyroids, as well as the thyroid. Furthermore, any residual tissue may hypertrophy, resulting in incomplete hormone removal. Injection of a single dose of radioactive iodine (I^{131}) on the day of birth results in the destruction of the thyroid, but the nonspecific effects of exposure to radioactive material must be considered. While these problems are not insurmountable, it was not feasible to include these treatment groups in the present investigation.

Method

Subjects

Charles River CD strain rats (timed pregnant) were used throughout the investigation. They were received in the laboratory between the 12th and 15th day of gestation, at which time the females were randomly assigned to one of the four treatment groups. Their litters served as subjects in the analysis of home orientation development, which was studied concurrently with maternal behavior.

Table 1 indicates the final number of litters in each treatment group, the percentage of pups surviving through day 22 in each group, and the final number of pups surviving through day 22.

Maintenance

All of the subjects were housed in 32.8 x 37.8 x 16.8 cm polycarbonate cages, located in a small animal room in the Animal Institute, Albert Einstein College of Medicine. The ambient temperature ranged between 21.1°C and 26.7°C, but for the most part remained between 22.2°C and 23.3°C. Room lights were on from 7:00 A.M. to 7:00 P.M., and all testing was conducted between 8:00 A.M. and 6:00 P.M. Food and water were present *ad lib*. .

Eight cups of fresh shavings were placed in the cage when the females first arrived in the laboratory. The bedding was not changed until after the pups were born. On the day of birth, approximately one third to one half of the soiled shavings were replaced with fresh shavings. Then, between days 4 and 12 of the litter period, one third of the bedding was replaced with fresh shavings every other day, immediately following orientation testing. Between days 14 and 22, one half of the bedding was replaced every other day.

Procedure

General

Starting on day 15 of pregnancy, females assigned to the hypothyroid or replacement therapy treatments were placed on a goitrogenic diet of 0.2% propylthiouracil

Table 1

Number of litters and pups used
in each treatment

Treatment	Number of Litters at Start of Experiment	Number of Litters w/ >3 pups at Day 22	Number of Pups at Start of Experiment[a]	Number of Pups Surviving to Day 22	Percentage of the Pups Surviving to Day 22	Final Number of Pups Used in Expt.[b]
Hypothyroid	10	7	56	31	55.4	27
Hyperthyroid	8	8	48	42	87.5	42
Replacement Therapy	10	9	57[c]	45	78.9	43
Control	8	8	47[d]	46	97.8	44

[a] Includes foster pups and pups in litters of less than 3 pups at day 22

[b] Excludes foster pups and pups in litters of less than 3 pups at day 22

[c] Includes one foster pup in each of two litters

[d] Includes two foster pups in one litter

(6-N-propyl-2-thiouracil, ICN Nutritional Biochemicals, Cleveland, Ohio), mixed with powdered Teklad rat and mouse diet. Antithyroid drugs such as methyl- and propylthiouracil act by preventing the iodination of tyrosine and hence thyroxine synthesis (Turner, 1971). These drugs seem to readily cross the placenta and also pass through the lactating female via the milk. Day 15 was chosen for the initiation of drug therapy since iodine uptake has been detected in the fetal thyroid at 18 days of gestation in the rat, and colloid-containing thyroid follicles and serum thyroxine are detected at this time (Swaab, 1975). In addition, Hamburgh, Sobel, Koblin and Rinestone (1962) have shown that maternal thyroxine passes through the placenta during the last third of gestation in the rat. By initiating PTU treatment on day 15, both fetal and maternal sources of thyroxine are eventually eliminated. Mothers of control and hyperthyroid litters were maintained on the Teklad diet without PTU.

Thyroxine solution was prepared by dissolving 2 mg thyroxine (L-thyroxine sodium, Smith Kline and French Laboratories, Philadelphia, Pennsylvania) in 26 ml 0.01 N NaOH and 74 ml 0.9% NaCl solution. Beginning on day 1, pups assigned to the hyperthyroid condition were injected daily as follows: day 1 to 7, 1 μg thyroxine; day 8 to 14, 2 μg thyroxine; and day 15 to 22, 4 μg thyroxine. Pups receiving replacement therapy were injected daily as follows: day 1 to 7, 0.8 μg thyroxine; day 8 to 14, 1.6 μg thyroxine; and day 15 to 22, 3.2 μg thyroxine. The hormone was injected subcutaneously in 0.05 to 0.20 ml of fluid. Control and hypothyroid pups were injected daily with equivalent amounts of physiological saline.

Beginning three days prior to the expected day of parturition, cages were checked daily at 10:00 A.M. for the presence of new litters. All new litters found on that check were considered day 1 pups. Litter size was reduced to six to minimize differences in maternal behavior due to differences in litter size and to reduce competition for lactation sites. An effort was made to maintain a balanced sex ratio. Pups which were considerably smaller than the litter average or which had poor color were eliminated.

If a female gave birth to a litter with fewer than six pups, same-age foster pups from litters receiving the same prenatal treatment were used to increase the litter size. Foster pups were only introduced on day 1 and after this day no pup replacements were made. Data from foster pups were not included in the results, however.

Pups were numbered from one to six on their dorsal surface with a nontoxic waterproof black marker. After day 14, pups were also marked on the tail, since markings on the fur became unreliable after this age.

Pups were weighed to the nearest 0.1 g on day 1 and then every other day between days 2 and 22, using an Ohaus Harvard trip balance (maximum capacity 2000 g, Ohaus Scale Corporation, Union, New Jersey) before day 14 and an Ohaus triple beam balance (maximum capacity 2160 g, Ohaus Scale Corporation, Union, New Jersey) between days 14 and 22. Pups were also examined daily for the presence of complete bilateral pinna unfolding and complete bilateral eye opening.

Data were collected in several replications. In any replication, at least one litter was assigned to each of the four treatments.

Maternal Behavior Analysis

The following maternal behaviors were assessed:

1) nesting
2) nursing, grooming of pups by mothers, and contact
3) retrieval of pups

Nest Rating

Approximately 18 to 20 hours before each nest was rated, the existing nest was completely destroyed and the shavings were scattered uniformly on the cage floor. The nest was rated at the same time each day for a given female, while the nests of all litters within a treatment group were rated at different times throughout the day. Nests were rated daily between days 2 and 22, using a scale devised by Seitz (1958):

0 - no nest
1 - shavings trampled down in one corner of the cage
2 - shavings pushed aside to make a bare spot in the corner of the cage
3 - low ring of shavings around a bare spot in the corner
4 - all shavings in the cage were piled into a high ring around a bare spot in the corner

Nursing, Grooming and Contact

Two 15 min observations were carried out starting on day 2 and continuing every other day until day 22. The first 15 min observation was made before disturbing the female and litter and the second observation was made approximately one hour after the female and pups were reunited following the retrieval test (see page 24), and after the weighing, marking and injecting of the pups. The observations of any given litter occurred at the same time each day, while the observations of all litters within a treatment group were randomly distributed through the day.

The 15 min observation period was divided into sixty 15 sec intervals and the following were noted on a check list during each interval:

1) nursing - the mother was considered to be nursing if she was crouched over the pups in the nest area or if at least 2 pups were suckling either beneath or alongside her _and_ she was not eating, drinking, nestbuilding or grooming herself.
2) grooming of pups - the mother was observed licking one or more of the pups.
3) contact - the mother was considered in contact with the pups if at least one pup was in direct contact with her body, excluding the tail. The number of pups not in contact with the mother at the end of each 15 sec interval was noted.

Retrieval

Females were given retrieval tests starting on day 2 and continuing every other day until day 14. Retrieval tests were performed immediately after the first 15 min observation. The home cages were modified to have a 9.0 cm wide door which could be raised to allow the female access to the apparatus used in retrieval testing. The door was situated in the center of one of the 32.8 cm walls.

A plexiglass retrieval alley, which attached to the home cage, was used for retrieval testing. The alley, which measured 33 x 72 x 26 cm high, was divided into two sections by an opaque partition which could be raised about 10 cm to permit access to the entire alley. One section of the alley held the pups during retrieval testing and the other section served as a holding chamber for the female prior to testing. The floor of the retrieval alley, as well as the opaque partition, was covered with black matte finish contact paper.

The retrieval alley was attached to the home cage and the home cage door was then raised, allowing the mother to move into the holding section. If she did not enter within 2 min, she was picked up and placed in the holding section. After the mother had entered the alley, the home cage door was lowered and the pups were removed to the far end of the retrieval alley. After 2 min, the female was allowed access to the home cage and after an additional 2 min the opaque partition was raised so that the pups were available for retrieval. The latency to contact the first pup, as well as the latency to retrieve each pup into the home cage, was noted. Testing was terminated when all pups were retrieved, or when 5 min had passed.

Summary of Procedure

To summarize, for a given litter, the nest was rated and the litter was then observed for 15 min. At the end of the observation, maternal retrieval was assessed. A second observation was made one hour after the female and pups were reunited.

Results

The means and standard errors are presented in the Appendix. The data were analyzed using a one-way analysis of variance with repeated measures. Because of the unequal number of litters in each group, post-hoc comparisons were made using the Scheffé method.

There were no significant differences in the number of pups born to females belonging to the hypothyroid, hyperthyroid, control or replacement therapy treatment groups. The females which received propylthiouracil (PTU) during pregnancy, i.e. mothers of hypothyroid litters and mothers of litters receiving replacement therapy, averaged 9.3 and 7.3 pups per litter, respectively, while mothers of control and hyperthyroid litters averaged 8.6 and 8.8 offspring per litter, respectively ($\underline{F} = 0.89$, $\underline{df} = 3/28$, $p > 0.05$). There were considerable differences in pup mortality during the litter period, however. Control litters had a survivorship through day 22 of 97.8%; hyperthyroid litters had a rate of 87.5%; and litters receiving replacement therapy had a survivorship of 78.9%. In contrast, hypothyroid litters had a survivorship through day 22 of only 55.4%, with none of the pups surviving to maturity.

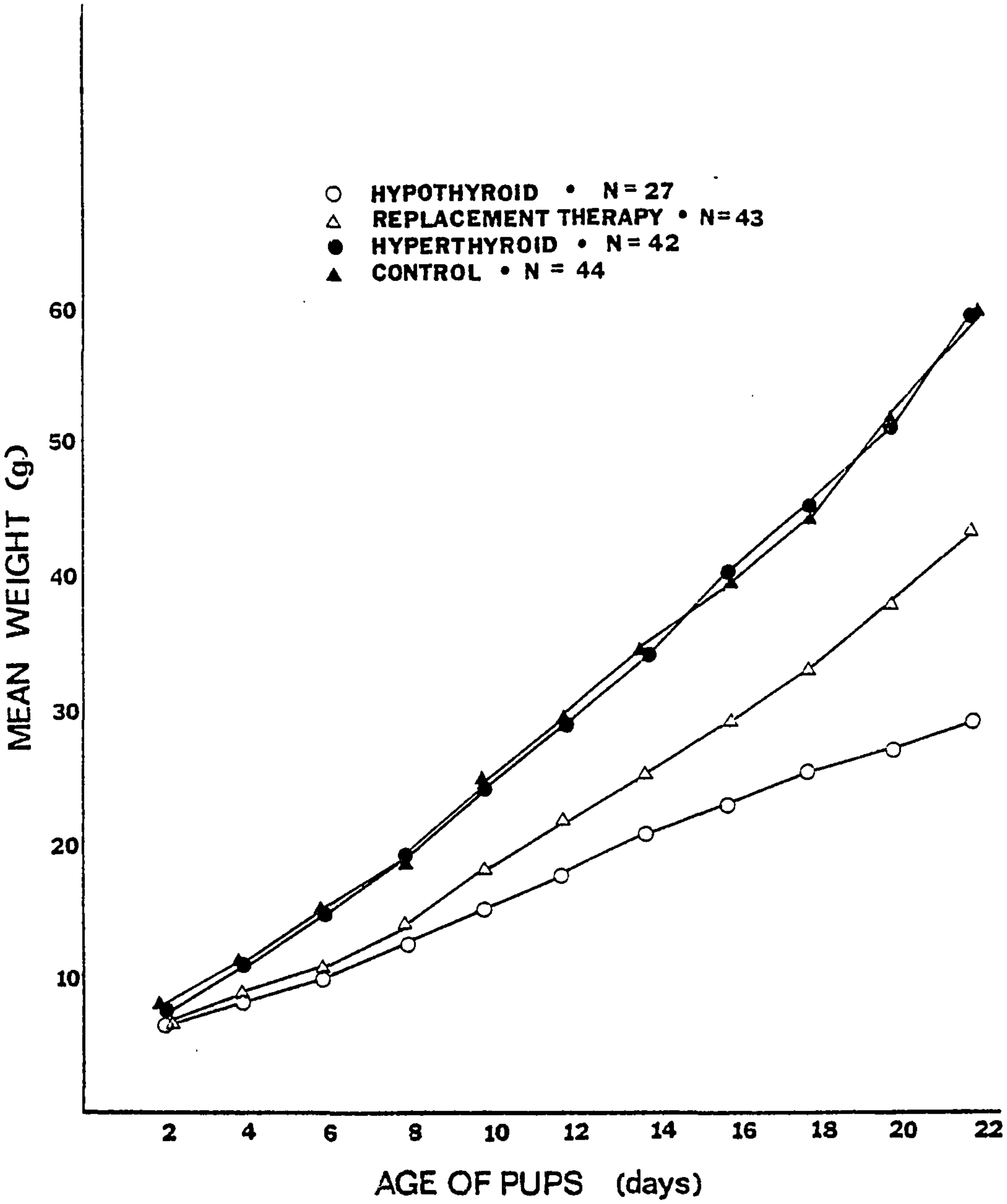
Physical Development of the Pups

Weight

The birth weights of litters whose mothers had received PTU from day 15 of gestation, i.e. hypothyroid litters and litters receiving replacement therapy, were 5.7 g (± 0.2 S.E.) and 5.7 g (± 0.3), respectively, while litters not treated prenatally, i.e. control and hyperthyroid litters, had mean birth weights of 6.5 g (± 0.1) and 6.7 g (± 0.3), respectively. These differences in birth weight among the treatment groups were significant ($\underline{F} = 4.99$, $\underline{df} = 3/28$, $p < 0.001$). Post-hoc comparisons revealed that the birth weights of replacement therapy and hypothyroid pups differed significantly from the birth weights of hyperthyroid litters (Scheffé, $p < 0.05$).

As can be seen in Figure 1, there were considerable differences in body weight at later ages as well. The weight differences among treatments became more pronounced as pups grew older, and this age by treatment interaction was highly significant ($\underline{F} = 26.67$, $\underline{df} = 33/308$, $p < 0.001$). The hyperthyroid pups did not differ from the controls at any age, while the hypothyroid and replacement therapy pups were considerably lighter than controls. Hypothyroid litters differed significantly from control litters on days 16 through 22 and differed from hyperthyroid litters on days 18 to 22 (Scheffé, $p < 0.05$). The litters receiving replacement therapy were heavier than the hypothyroid litters. This difference between hypothyroid and replacement therapy litters

Figure 1. Mean weights (in grams) of hypothyroid, hyperthyroid, replacement therapy and control pups, as a function of pup age. Means and standard errors are presented in the Appendix, Table A.


○ HYPOTHYROID • N = 27
△ REPLACEMENT THERAPY • N = 43
● HYPERTHYROID • N = 42
▲ CONTROL • N = 44
MEAN WEIGHT (g.)
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
AGE OF PUPS (days)

became pronounced by days 12 to 14. By day 22, animals receiving replacement therapy were approximately 26% lighter than controls, while hypothyroid pups were 51% lighter. The age effect was highly significant (F = 1076.77, df = 11/308, $p < 0.001$), as was the treatment effect (F = 25.35, df = 3/28, $p < 0.001$).

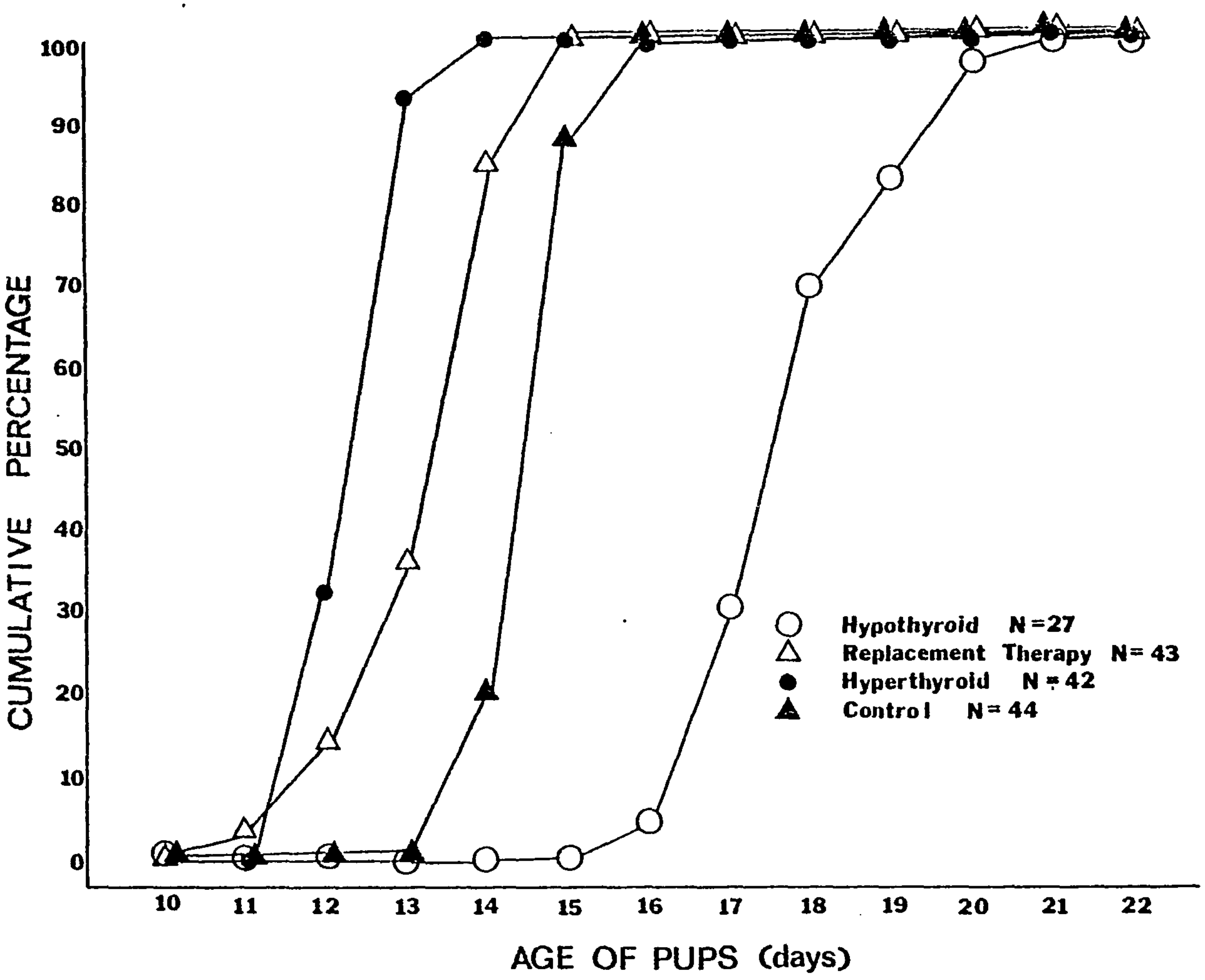
Pinna Unfolding

Pinna unfolding occurred most frequently on days 3 and 4, with no significant difference among the pups in the four treatment groups (F = 2.19, df = 3/28, $p > 0.05$).

Eye Opening

Pup hormonal state had a considerable effect on the day of bilateral eye opening. As can be seen in Figure 2, control pups showed the highest frequency of eye opening on day 15 and by day 16, 100% of control pups showed eye opening. Hyperthyroid pups most frequently showed eye opening on day 13 and by day 14, 100% had their eyes open. Day 14 was the day on which the largest number of pups receiving replacement therapy showed bilateral eye opening, and 100% of these pups had both eyes open by day 15. In contrast, hypothyroid pups most frequently showed eye opening on day 18 and not until day 21 did 100% of these pups show complete bilateral eye opening. The cumulative percentage curves are remarkably similar in shape and appear to be simply displaced from each other along the abscissa. Khamsi and Eayrs (1966) found a linear relationship between day of eye opening and the log daily dose of thyroid hormone received, suggesting that eye opening may serve as a fairly sensitive indicator of level of

Figure 2. Cumulative percentage of subjects showing complete bilateral eye opening as a function of pup age.


CUMULATIVE PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
10
11
12
13
14
15
16
17
18
19
20
21
22
AGE OF PUPS (days)
○ Hypothyroid N=27
△ Replacement Therapy N= 43
● Hyperthyroid N = 42
▲ Control N= 44

thyroid hormones. The animals receiving thyroxine replacement therapy may therefore be slightly hyperthyroid, since eye opening was somewhat accelerated in these litters relative to controls. The four treatment groups differed significantly ($\underline{F} = 68.6$, $\underline{df} = 3/28$, $p < 0.001$). When pairwise comparisons were made, only the hyperthyroid treatment group and the replacement therapy treatment group did not differ significantly; all other pairwise comparisons revealed significant differences (Scheffé, $p < 0.05$).

Maternal Behavior

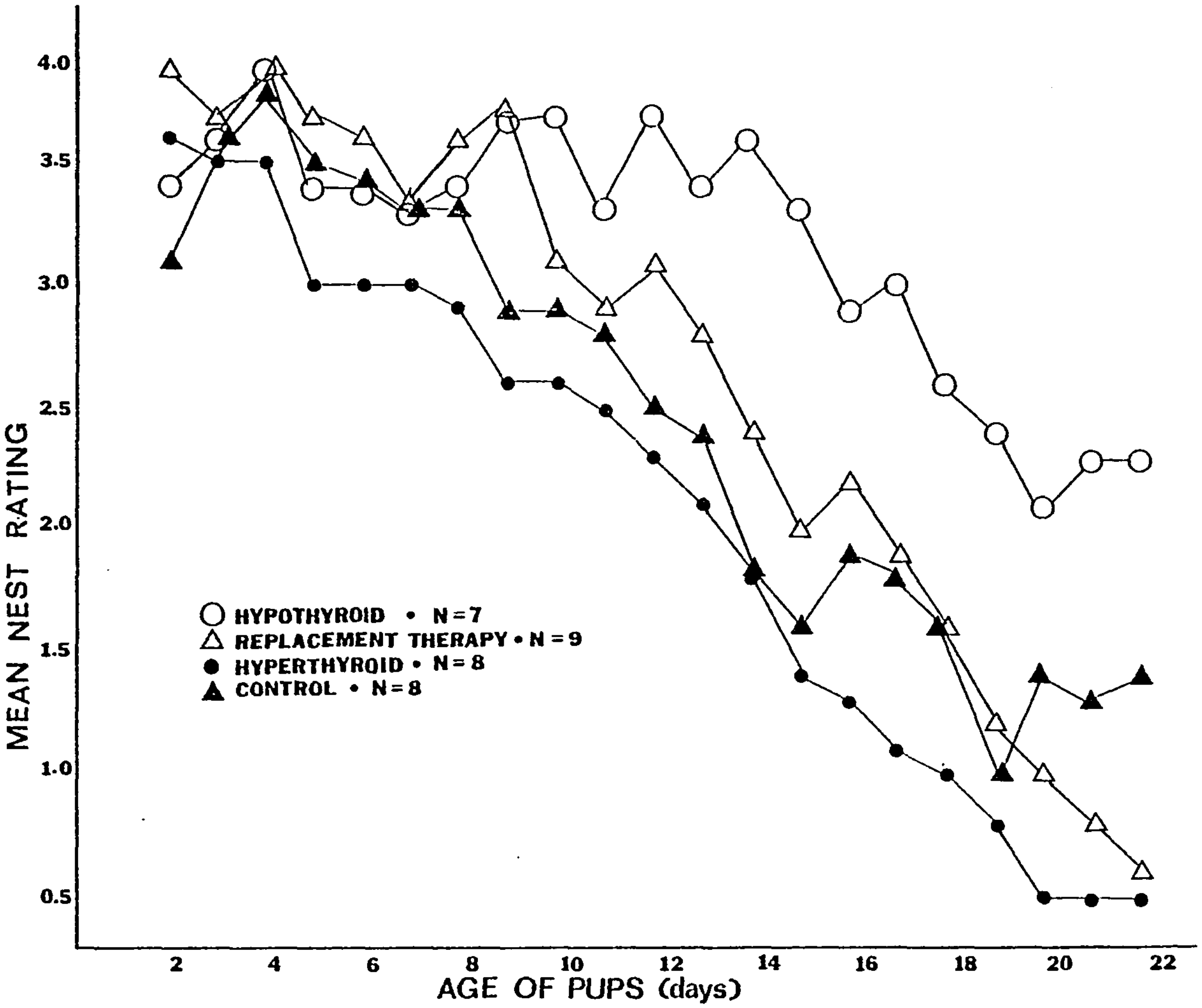
Maternal behavior data from litters in which less than three pups survived to day 22 were not included in the results.

The Scheffé tests failed to reveal any significant differences between treatment groups when pairwise comparisons were made. As a result, statements about differences between treatment groups are based on inferences from the data and must be considered tentative in the absence of statistical support.

Nest Rating

There were marked differences in the quality of the nest among the litters from the four treatment groups, as can be seen in Figure 3. Early in the litter period, there were no differences among the four treatments in the mean nest rating, with litters in all groups having high quality nests. By day 10, there were marked differences among the treatments. The nests of hypothyroid litters were maintained in better condition for a longer period of time than the

Figure 3. Mean nest rating as a function of pup age. Means and standard errors are presented in the Appendix, Table B.


MEAN NEST RATING
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2
4
6
8
10
12
14
16
18
20
22
AGE OF PUPS (days)
○ HYPOTHYROID • N=7
△ REPLACEMENT THERAPY • N=9
● HYPERTHYROID • N=8
▲ CONTROL • N=8

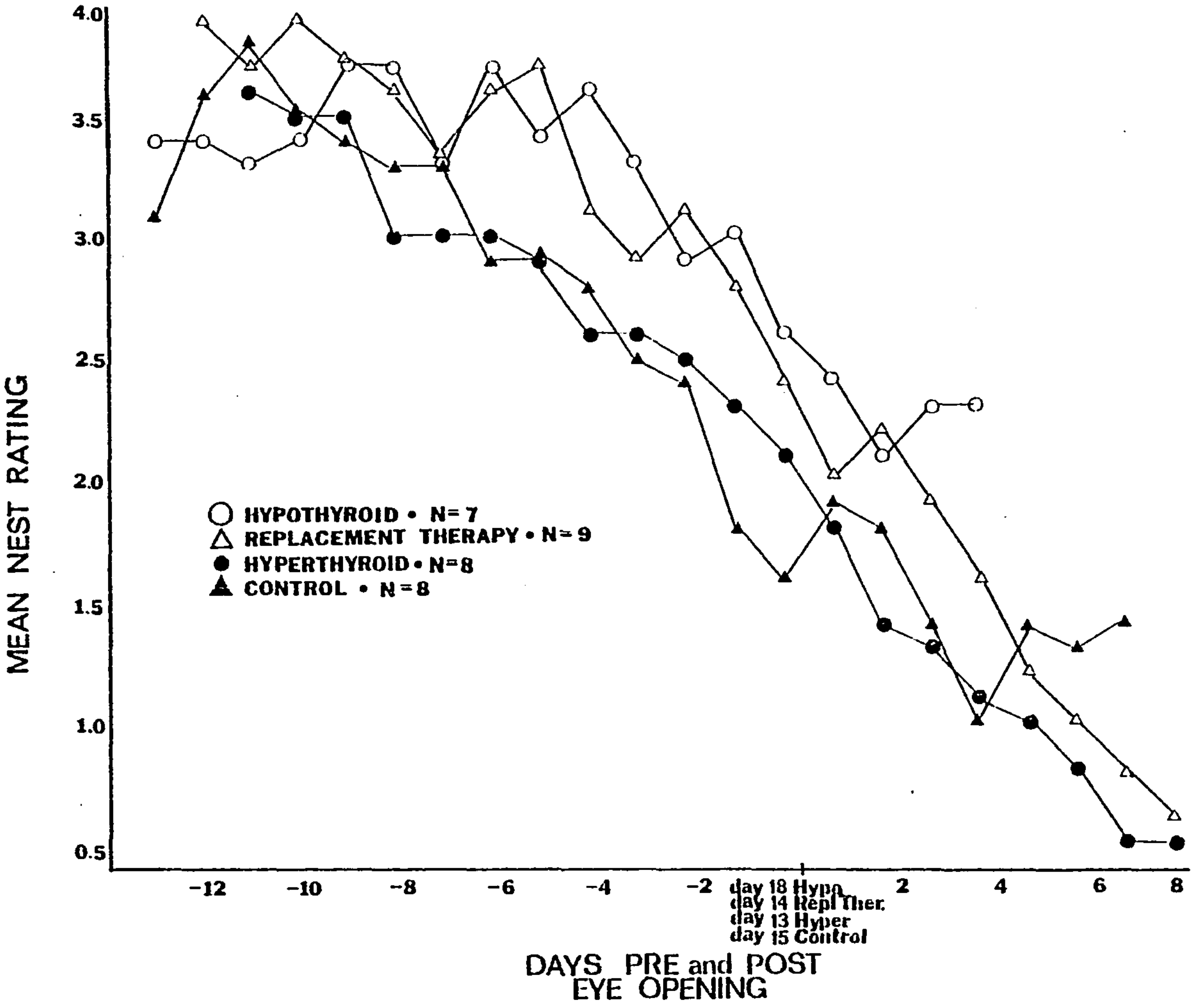
nests of other litters. It also appears as if nests of hyperthyroid litters showed a more rapid decline in quality than nests in control litters, and nests from replacement therapy litters showed a less rapid decline than controls. Variance analysis revealed a significant effect of pup age (F = 59.21, df = 20/560, $p < 0.001$), a significant treatment effect (F = 20.00, df = 3/28, $p < 0.001$) and a significant interaction between age and treatment (F = 2.37, df = 60/560, $p < 0.001$).

These data suggest that both maternal and pup hormonal conditions influence the decline in nest quality. If only the maternal condition were influencing nesting, one would expect mothers of hypothyroid and replacement therapy litters to behave similarly, since both classes of females received PTU. Likewise, mothers of control litters and mothers of hyperthyroid litters should behave identically, since their hormonal condition was presumably the same, i.e. normal. The data do not support either of these predictions. If pup hormonal condition were solely responsible for the decline in nesting, one might expect the nest quality of replacement therapy litters to decline somewhat faster than in control litters, since on the basis of the eye opening data it seems as if these pups may have been somewhat hyperthyroid. Instead, it was found that nest quality declined less quickly in replacement therapy litters than in controls.

In order to further analyze these findings, the mean nest ratings were plotted as a function of the number of days pre- and post- eye opening. Hence, day 0 on Figure 4 represents the modal day of eye opening for the different types of litters (i.e. day 18 for hypothyroid pups, day 15 for control pups, day 14 for replacement therapy pups and day 13 for hyperthyroid pups). This treatment of the data had the effect of "equating" pups in terms of eye opening, but it should be noted that other measures of development may still differ among treatments. In fact, by equating hyperthyroid and control litters in terms of eye opening, these litters were made to differ in terms of body weight, since these pups differed in day of eye opening, but not in body weight (see Figures 1 and 2). The choice of eye opening as a developmental landmark is thus admittedly somewhat arbitrary but apparently not completely unjustified.

Even when pup development was equated in this fashion, significant differences in nest ratings were still found among treatments ($\underline{F} = 8.48$, $\underline{df} = 3/28$, $p < 0.001$). On the basis of visual inspection of the curves in Figure 4, there appear to be two classes of mother in the four treatment groups. There was no difference between control mothers and mothers of hyperthyroid pups in mean nest rating; likewise, there seems to be no difference in nest rating between mothers of hypothyroid litters and mothers of litters receiving replacement therapy. However, beginning approximately one week before eye opening, there were clear differences in nest quality between mothers receiving PTU (i.e. mothers of hypothyroid and replacement therapy pups) and those not receiving PTU (i.e. mothers of control and hyperthyroid pups).

Figure 4. Mean nest rating as a function of the number of days pre- and post- eye opening.


MEAN NEST RATING
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
○ HYPOTHYROID • N= 7
△ REPLACEMENT THERAPY • N= 9
● HYPERTHYROID • N=8
▲ CONTROL • N=8
-12
-10
-8
-6
-4
-2
day 18 Hypo.
day 14 Repl Ther.
day 13 Hyper
day 15 Control
2
4
6
8
DAYS PRE and POST
EYE OPENING

Nursing, Grooming and Contact

Except where specified, there was no marked difference in the data obtained from the two observations (i.e. undisturbed and disturbed). For this reason, the results reported for nursing, grooming and contact are based on an average of the two observations.

Nursing

Nursing behavior appeared to be affected by hormonal treatment. As can be seen in Figure 5, there appear to be no differences in the percentage of intervals in which the female was observed in the nursing position among the mothers of control, hyperthyroid and replacement therapy pups, while mothers of hypothyroid pups were observed to be in the nursing position more often than mothers of other pups after days 10 to 12. Variance analysis indicates a significant age effect ($\underline{F} = 23.51$, $\underline{df} = 10/280$, $p < 0.001$), a significant treatment effect ($\underline{F} = 6.56$, $\underline{df} = 3/28$, $p = 0.002$) and a significant Age x Treatment interaction ($\underline{F} = 1.7$, $\underline{df} = 30/280$, $p = 0.015$). When pup development was "equated" on the basis of eye opening, it appears as if the differences among the four groups disappear (see Figure 6). Visual inspection of the figure seems to support the conclusion that differences among the mothers were based on differences in physical and behavioral characteristics of the pup which were correlated with eye opening. However, because observations were made only every other day, the day of eye opening was not necessarily a day on which maternal behavior was observed. Variance analysis of the data was not possible,

Figure 5. Mean percentage of intervals during which nursing occurred in hypothyroid, hyperthyroid, replacement therapy, and control litters as a function of age. Means and standard errors are presented in the Appendix, Table C.

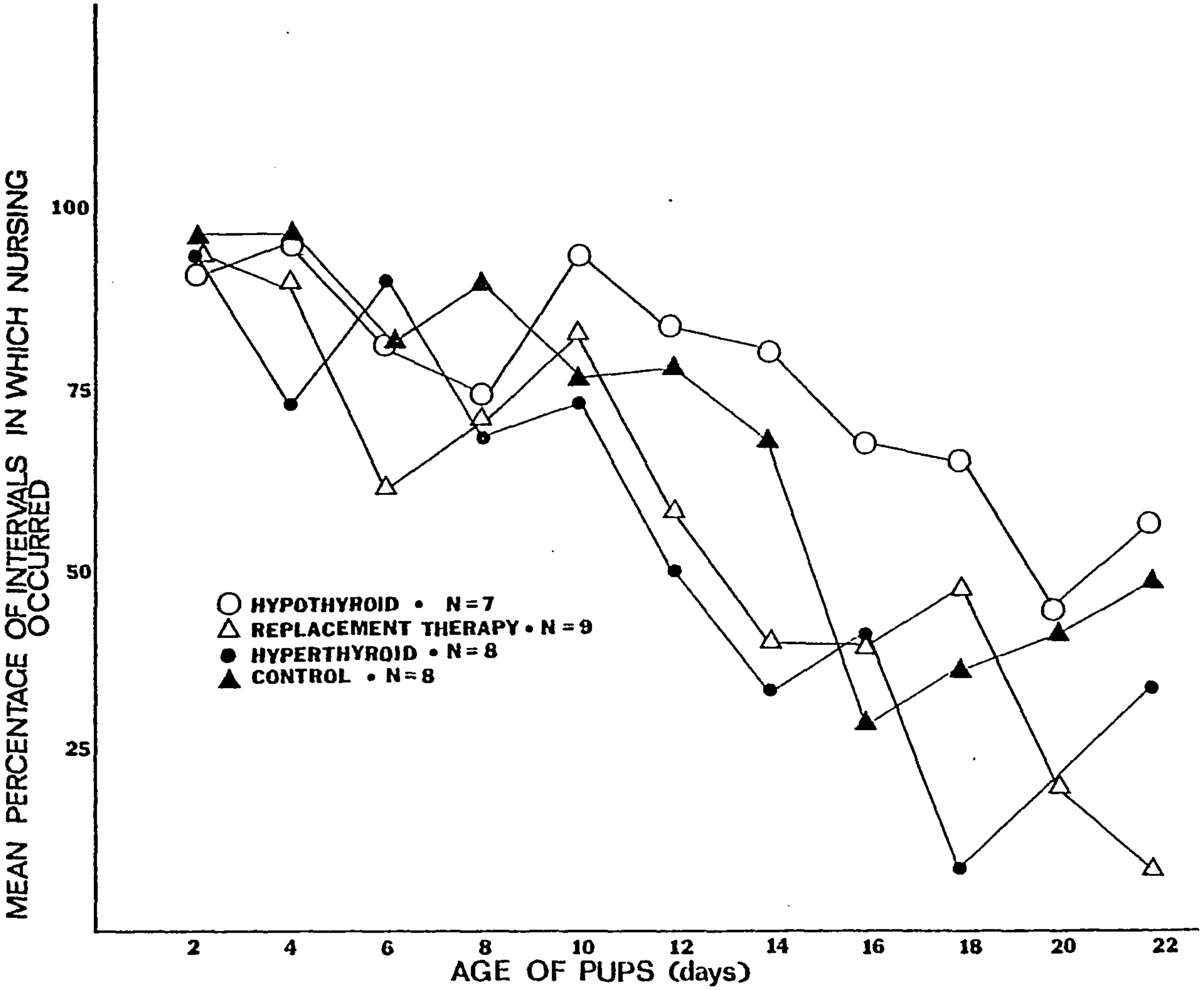
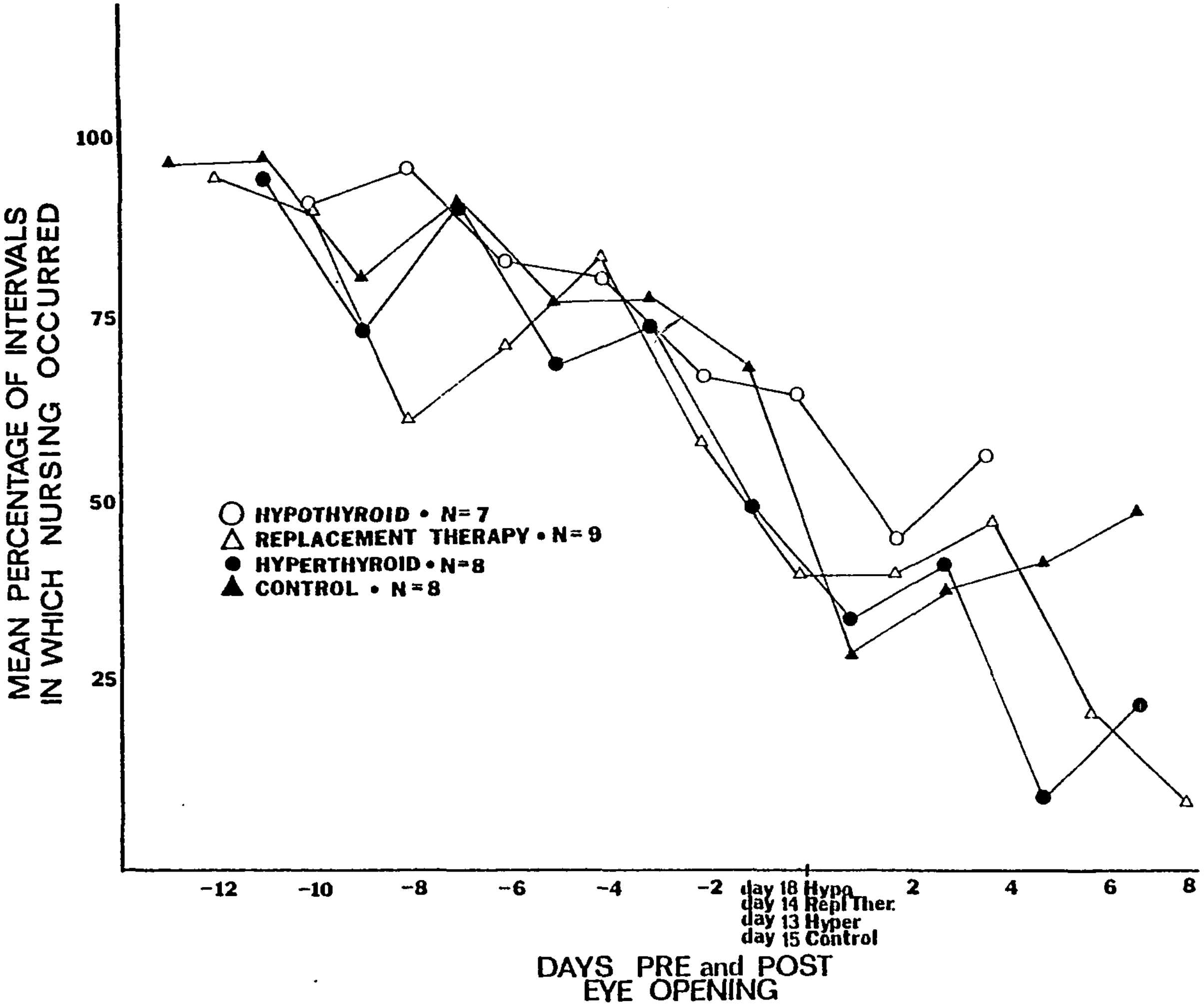

MEAN PERCENTAGE OF INTERVALS IN WHICH NURSING OCCURRED
100
75
50
25
○ HYPOTHYROID • N=7
△ REPLACEMENT THERAPY • N=9
● HYPERTHYROID • N=8
▲ CONTROL • N=8
2
4
6
8
10
12
14
16
18
20
22
AGE OF PUPS (days)

Figure 6. Mean percentage of intervals during which nursing occurred, as a function of the number of days pre- and post- eye opening.


MEAN PERCENTAGE OF INTERVALS
IN WHICH NURSING OCCURRED
100
75
50
25
○ HYPOTHYROID • N=7
△ REPLACEMENT THERAPY • N=9
● HYPERTHYROID • N=8
▲ CONTROL • N=8
-12
-10
-8
-6
-4
-2
day 18 Hypo
day 14 Repl Ther.
day 13 Hyper
day 15 Control
2
4
6
8
DAYS PRE and POST
EYE OPENING

therefore, and the conclusions should be considered tentative.

Grooming

An analysis of the frequency of maternal grooming of pups led to an interesting, but somewhat surprising, finding. Mothers of hypothyroid and replacement therapy litters maintained a fairly constant, low level of grooming throughout the litter period. In contrast, mothers of hyperthyroid and control pups showed a peak in grooming behavior at 10 to 12 days for controls and at 6 to 10 days for hyperthyroid litters. These peaks were slightly exaggerated during the distrubed observations for both types of mothers, although at ages before or after the peak, there was no difference between the disturbed and undisturbed observations. Variance analysis of the data indicate no significant treatment effect ($\underline{F}$ = 1.59, $\underline{df}$ = 3/28, $p = 0.214$), but there was a significant age effect ($\underline{F}$ = 6.27, $\underline{df}$ = 10/280, $p < 0.001$) and a significant interaction between age and treatment ($\underline{F}$ = 2.08, $\underline{df}$ = 30/280, $p = 0.001$).

Contact

There were small but significant differences in the mean number of intervals in which mother-young contact occurred among the four treatment groups, with mothers and pups in control and hypothyroid treatment groups remaining in contact more often than mothers and pups in the hyperthyroid and replacement therapy treatment groups. There was a significant treatment effect ($\underline{F}$ = 3.16, $\underline{df}$ = 3/28, $p = 0.040$) and a significant age effect ($\underline{F}$ = 8.09, $\underline{df}$ = 10/280,

$p < 0.001$), as well as a significant Age x Treatment interaction ($\underline{F} = 1.69$, $\underline{df} = 30/280$, $p = 0.017$).

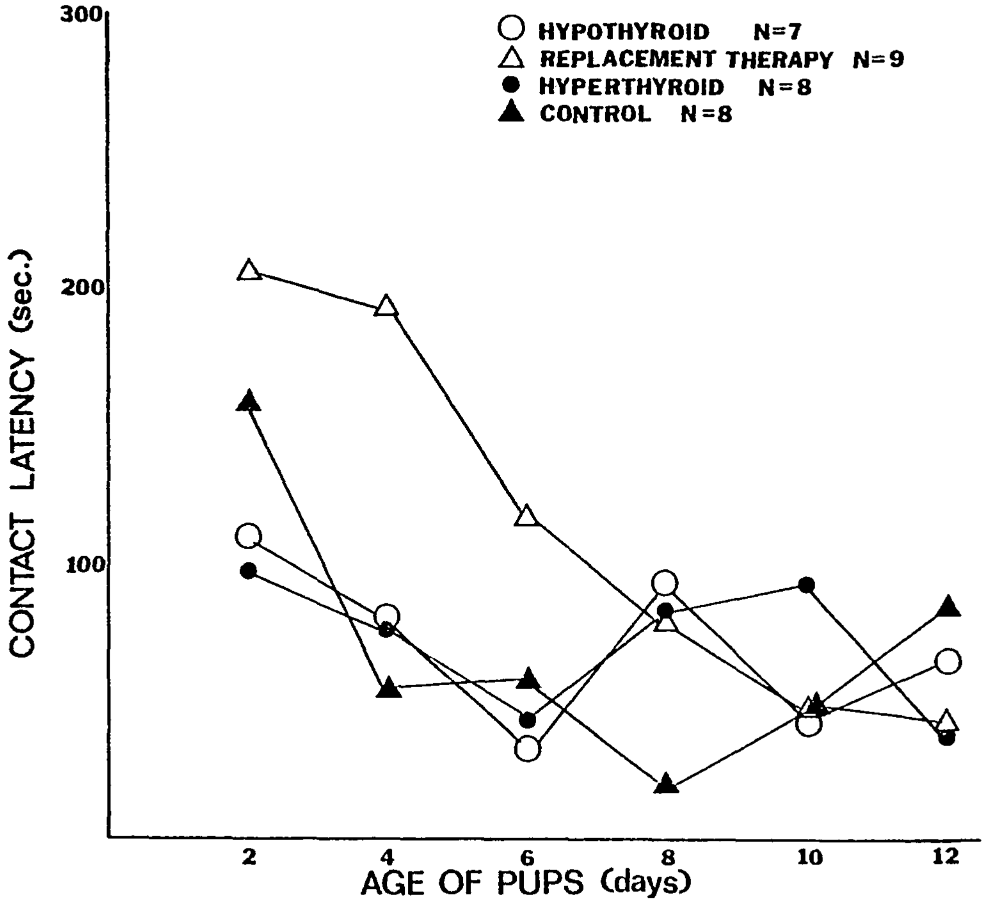
There were also differences among the four groups in the mean percentage of pups not in contact with the female. In all treatments, there was a gradual increase in the percentage of pups not in contact. On day 2, over 90% of the pups were in contact with the female, but this percentage decreased with advancing age of the pups. By days 16 to 18, approximately 60% of the pups from hyperthyroid, control and replacement therapy litters were not in contact with the female during an observation period, but only 30% of the hypothyroid pups were not in contact. Even by days 20 to 22, only 45% of the hypothyroid pups were not in contact with the female. The differences among treatments was significant ($\underline{F} = 4.25$, $\underline{df} = 3/28$, $p = 0.014$), as was the age effect ($\underline{F} = 18.74$, $\underline{df} = 10/280$, $p < 0.001$) and the Treatment x Age interaction ($\underline{F} = 1.53$, $\underline{df} = 30/280$, $p = 0.041$).

Retrieval

Contact Latency

Figure 7 shows the mean latency to contact the first pup during the retrieval test, as a function of pup age. The mothers of litters receiving replacement therapy showed longer latencies on days 2, 4 and 6 than mothers of other pups, and after this time there were no differences among the mothers in the four treatment groups, producing a significant Treatment x Age interaction ($\underline{F} = 2.04$, $\underline{df} = 15/140$, $p = 0.017$). A significant age effect was also found ($\underline{F} = 5.86$, $\underline{df} = 5/140$, $p < 0.001$), but there was no significant

Figure 7. Mean latency to contact the first pup during the retrieval test, as a function of pup age. Means and standard errors are presented in the Appendix, Table G.


○ HYPOTHYROID N=7
△ REPLACEMENT THERAPY N=9
● HYPERTHYROID N=8
▲ CONTROL N=8
CONTACT LATENCY (sec.)
300
200
100
AGE OF PUPS (days)
2
4
6
8
10
12

treatment effect (F = 0.87, df = 3/28, p = 0.47). When the latency data were computed to include only those females which did contact the pups, there were very few differences in the latency to contact pups among mothers of control, hyperthyroid and hypothyroid litters, while mothers of replacement therapy litters showed long contact latencies on days 6 and 8. If the percentage of females contacting pups is examined, it becomes clear that mothers of replacement therapy litters were somewhat less likely to contact pups at early ages. Since the first contact latency is likely to be long, it is possible that the peak in latency at days 6 and 8 for mothers of litters receiving replacement therapy reflects the fact that some of the females were contacting pups for the first time on these days.

Retrieval Latency

There were no differences among the four treatment groups in the latency to retrieve the first pup (F = 1.77, df = 3/28, p = 0.177), although there were significant changes with age (F = 3.49, df = 5/140, p = 0.005). There was also no interaction between treatment and age (F = 0.77, df = 15/140, p = 0.713).

Since the number of pups in each litter varied, the total time to retrieve the entire litter was not analyzed.

Summary of Results

1) Mothers of hypothyroid litters maintained high quality nests for a longer period of time during the litter period. They also showed higher levels of nursing and contact with pups.

2) Mothers of hyperthyroid and control litters showed a peak in grooming during the early litter period. This peak was not shown by mothers receiving PTU (i.e., mothers of hypothyroid litters and litters receiving replacement therapy).

3) Mothers of hyperthyroid pups showed an earlier decline in nest quality than mothers of control litters.

4) Mothers of replacement therapy litters showed significantly longer latencies to contact their pups than mothers in other treatment groups. However, there were no differences among the treatment groups in latency to retrieve the first pup.

Discussion

The mothers assigned to the four treatment groups showed substantial differences in maternal behavior. Depending on the response measured, some differences were due to maternal hormonal condition, some to the hormonal condition of the offspring, and some were influenced by both maternal and pup hormonal condition.

Nests in hypothyroid litters were maintained in better condition for a greater part of the litter period than in control litters, while in hyperthyroid litters there was an accelerated decline in nest quality (see Figure 3). The litters receiving replacement therapy were quite possibly hyperthyroid, since eye opening was accelerated relative to controls in these pups. Nevertheless, replacement therapy litters had slightly better nests than control litters. This suggests that the maternal hormonal condition may be a factor in determining the level of nest quality. When the mean nest rating was examined as a function of days pre- or post- eye opening (see Figure 4), some of the differences among treatments disappeared. By "equating" pup development on the basis of eye opening, differences in nest quality between control and hyperthyroid litters disappeared, as did differences among hypothyroid litters and litters receiving replacement therapy. However, there was a significant difference in the quality of nest in litters where the mothers were receiving PTU (i.e. hypothyroid litters and litters receiving replacement therapy) and in litters where the mothers were not receiving PTU (i.e. control and hyperthyroid litters). It seems as if mothers receiving PTU may maintain slightly better nests than mothers not receiving PTU starting one week before the modal day of eye opening for their litters. This suggests that the mother's hormonal state was affecting the quality of the nest, since mothers receiving PTU were most likely hypothyroid themselves. Richter (1941) found that removal of the thyroid resulted in increased nesting behavior in rats. It is possible that maintenance of the females on 0.2% PTU is producing a hypothyroid state in the female which is sufficient to alter her nesting behavior. Unfortunately, no measures were taken of maternal thyroxine levels to determine the extent to which the mothers of hypothyroid and replacement therapy litters were themselves hypothyroid.

Pup condition is also important in affecting nest quality. Hyperthyroid litters showed a more rapid decline in nesting than controls, although maternal hormonal state was identical. Likewise, mothers of hypothyroid pups maintained high quality nests longer than mothers of replacement therapy litters, although mothers of both types of litters were receiving PTU. The mechanism by which the condition of the pups is affecting nest quality is unclear. It is possible that the body temperature of the pups is important in affecting the behavior of the mother, since hypothyroid pups develop thermoregulation considerably later than controls (Hamburgh, 1968). In fact, although no quantitative measure was taken, the 10 to 12 day hypothyroid pups in the present study felt cooler to the touch after being out of the nest for 30 to 60 min than did controls, while hyperthyroid pups felt warmer than controls. If the lactating females respond to changes in pup temperature, this might explain the observed differences in nest building. The pups might also be influencing the quality of nest directly, by differences in the amount of activity. Hyperthyroid animals became active sooner than controls, possibly contributing to an earlier destruction of the nest, while hypothyroid pups were relatively inactive, possibly permitting the nest to be maintained in good condition longer than normal. Perhaps a more direct measure of maternal nest building could distinguish between the contributions of mother and pup behavior to the maintenance of the nest.

At any rate, the nest is maintained in good condition in hypothyroid litters for a longer period of time during the litter period than normal. This may serve to maintain the pup's body temperature, as would the higher levels of nursing and general contact received by these pups.

Considerable differences were also found in the amount of nursing (see Figure 5), grooming and mother-young contact. Until day 10, there was no difference among the females in the mean percentage of intervals in which the females was observed in the nursing position. Mothers of hyperthyroid, replacement therapy and control pups showed an earlier decline in nursing than did mothers of hypothyroid pups. These differences appear to be due to differences in pup condition. When pups were "equated" in terms of day of eye opening, the differences among the treatments disappeared (see Figure 6). These results are consistent with Nicoll and Meites (1959), who found that the decline in lactation could be delayed by replacing a female's own pups with an equal number of very young pups. The immature appearance or behavior of the hypothyroid pups might thus be important in maintaining the high levels of nursing. The mothers and pups in the hypothyroid treatment group showed greater amounts of general mother-young contact than mothers and pups assigned to the hyperthyroid or replacement therapy treatment groups. Furthermore, mothers of hypothyroid litters were observed to be in contact with a larger percentage of their litter than control mothers after days 8 to 10. The hypothyroid pups may have contributed to the increased amounts of contact with the mother by leaving the nest area at a later age than controls. These differences in general mother-young contact may reflect or may give rise to the pronounced differences in nursing behavior which was observed.

It should be pointed out that the observed differences in "nursing" were actually differences in the percentage of time the females were observed to be in the nursing position, and not necessarily differences in the amount of suckling by pups. Of course, this increased contact with the mother would increase the opportunity for suckling by pups, but further studies would have to be made to determine whether hypothyroid pups actually do spend more time suckling.

Maternal hormonal state affected the grooming of pups. Mothers of replacement therapy and hypothyroid litters showed low, constant levels of grooming throughout the litter period, while the mothers of control and hyperthyroid animals showed large peaks in the amount of grooming between days 6 and 12. It seems as if hypothyroidism in the female affected her ability to perceive or respond to changes in the pup at these ages. There is some evidence that both taste and smell deficits are common in human patients suffering from hypothyroidism (McConnell, Menendez, Henkin and Rivlin, 1975). These investigators also report that reversible deficits in taste perception were observed in rats which were radiothyroidectomized as adults. It is possible that deficits in the ability to taste or smell their pups were responsible for the low levels of grooming on days 6 to 12 by mothers receiving PTU.

There were only small differences in maternal retrieval of pups among the four treatment groups. The mothers of litters receiving replacement therapy showed significantly longer latencies to contact their pups on the first few days of testing. It is not clear why these differences exist only in these mothers, but since there were no significant differences in retrieval latency, the differences in contact latency seem to not be significant for the occurrence of normal retrieval behavior. The absence of treatment differences in retrieval behavior may be due to the fact that retrieval is not necessarily a measure of maternal behavior. Plume, Fogarty, Grota and Ader (1968) found that lactating, pregnant and nonpregnant female and adult male rats all showed retrieval of both inanimate objects and live rat pups. Lactating females showed a marked preference for the pup over the inanimate object, but the other groups showed no such preference. It is possible that the absence of effects on retrieval behavior in the present investigation may be due to the fact that retrieval of objects occurs ubiquitously in the rat. Finally, there is no reason to expect that all measures of maternal behavior will be affected similarly. The underlying organization and motivational basis for different maternal behaviors may be quite different, thus affecting the manner in which the behaviors are affected by hormonal treatment of mother or pup.

In general, it appears as if hypothyroid pups received adequate maternal care. The nest of the hypothyroid litter was maintained in better condition than the nest of a control litter, which might compensate for the deficits in thermoregulation shown by these pups. The increased percentage of time in which the female was observed in the nursing position, in addition to the increased amount of general contact, might also compensate for the deficiency in thermoregulation in these pups, as well as provide increased nursing opportunities for the young. Furthermore, there were no differences in maternal retrieval of pups between mothers of hypothyroid litters and mothers of control litters. From these measures of maternal behavior, it appears as if the hypothyroid pups received fully adequate maternal care. In fact, their mothers showed higher levels of nesting, nursing and contact, which would compensate for the delayed development of these pups. Mothers of hypothyroid offspring did show low levels of grooming of pups and there is a possibility that this deficit has an effect on pup behavior. However, mothers of replacement therapy litters showed a similar deficit, yet the behavior of their offspring was very similar to the behavior of control animals. It is therefore unlikely that the deficit in grooming shown by mothers of hypothyroid litters was responsible for the gross differences in behavior observed in these pups.

There is a possibility that the greater amounts of mother-young interactions in hypothyroid litters are responsible for some of the differences in behavior shown by hypothyroid pups. For example, it is possible that maintenance of the nest in good condition makes it less likely that the pups will leave the nest, thus preventing the development of home orientation at the normal time. However, it is considered unlikely that the enormous changes in brain morphology and adult "learning" ability are due solely to the differences observed in maternal behavior. It is more probable that the higher levels of mother-young interaction alleviate some of the severe effects of hypothyroidism. For example, maintenance of high quality nests and the high levels of nursing and contact would help to compensate for the delayed development of homeothermy in hypothyroid pups.

Litters assigned to the hyperthyroid treatment group generally showed a more rapid decline in nest quality, and perhaps nursing, than litters assigned to the control treatment group. It is possible that the early decline in maternal behavior in these litters is responsible for the accelerated emergence of some reflex responses and for some of the adult learning deficits previously reported in hyperthyroid animals. In the current investigation, some differences were observed between hyperthyroid and control pups. The hyperthyroid rats were indistinguishable from the controls on the basis of the home orientation test (see Section III, Development of Home Orientation), while on the gradient tests they developed the orientation response on an olfactory gradient sooner than controls (see Section IV, Development of Olfactory and Thermal Responsiveness). It is possible that the accelerated behavioral development is a result of the early decline in maternal behavior in their mothers. However, since the hormonal condition of the mothers of hyperthyroid and control litters was the same, it is more likely that the accelerated behavioral development of the pups was responsible for the early decline in maternal behavior. Of course, it is possible that the differences in maternal behavior could then interact with the initial behavior difference shown by the hyperthyroid pups to give rise to altered pup behavior at later ages.

With respect to the first question asked in the introduction of this paper, that is whether the severe effects of thyroid hormone manipulation may be mediated via differences in the quality of maternal care received, it would seem that this is not likely to be the case. In fact, it is possible that the maternal behavior shown by mothers of hypothyroid pups alleviates some of the severe deficits produced by perinatal hypothyroidism. However, further study of the role of maternal behavior in the development of hypothyroid and hyperthyroid rats would have to be made.

Perhaps one way to analyze the possible effects of the differences in maternal behavior shown by the mothers of hypothyroid and hyperthyroid litters would be to rear the pups artificially. In this way, the quality of care would be kept constant, so that any observed differences in behavioral development would most certainly not be due to differences in maternal behavior.

III. DEVELOPMENT OF HOME ORIENTATION

Introduction

It is apparent from an examination of the literature on the behavioral development of hormone treated pups that most of the work has concentrated on the emergence of various reflex responses. Very little attention has been paid to the more integrated behavioral patterns which young rats can show and which may be essential for their survival.

One well documented response that young rats show is an orientation response to the mother, to the other siblings and to the nest region. Scattering of pups outside the nest periodically occurs when they cling to the female's nipples as she leaves the nest area. During the early stages of the postnatal period, scattered pups are most often retrieved by the mother, but Bolles and Woods (1964) observed that orientation by pups to the mother and nest area can occur as early as 2 to 3 days after birth. Altman and Sudarshan (1975) also report successful nonambulatory orientation by day 3 and successful homing from some distance from the nest by day 8.

Turkewitz (1966) studied the development of home orientation in neonatal rats. In the homing test he used, animals began to respond to the nest as a preferred area of the home cage by day 13 and by day 17 showed a decline in homing. He suggests that homing in the rat pup is based on proximal stimuli, such as olfactory and thermal cues. With eye opening, visual stimuli become important in pup behavior, and may eventually elicit responses which compete with home orientation and which result in its decline after day 17.

Orientation to the home area has been studied in malnourished rat pups by Altman, Sudarshan, Das, McCormick and Barnes (1971), Altman, Brunner, Bulut and Sudarshan (1972) and Fleischer and Turkewitz (in press). Altman *et al.* (1971, 1972) report that severely malnourished rats show a deficiency in the ability to reach their sibs and also in the ability to reach their home cage, when they were displaced from the home. Fleischer and Turkewitz (in press) examined the development of homing in rat pups stunted by rearing in large litters. Pups from large litters showed a peak of 46% displaying homing by day 18, in contrast to controls, which showed a peak of 75% on day 14. Hence, the animals stunted by rearing in large litters showed a consistently lower level of homing than controls, as well as a delay in the peak percentage of pups showing homing. There was no attempt by either Altman *et al.* (1971, 1972) or Fleischer and Turkewitz (in press) to ascertain the basis of the impaired orientation, but Fleischer and Turkewitz suggest that there may be deficits in the development of olfactory responsiveness in the malnourished rat pups.

For the second portion of this investigation, hypothyroid, hyperthyroid, replacement therapy and control litters were tested during the litter period for the development of home orientation, in order to assess the development of an integrated behavior in these pups. The test used was similar to that used by Rosenblatt, Turkewitz and Schneirla (1969) in kittens and Fleischer and Turkewitz (in press) in malnourished rat pups, and tested the ability of hormone treated pups to make the integrated response of orienting to the home corner of the cage.

Method

Subjects

The subjects used in this portion of the investigation were the offspring of the females studied in the analysis of maternal behavior. The number of litters and the final number of pups in each treatment group are given in Table 1 (page 19).

Procedure

The general procedures for maintenance and hormone treatment were described in the previous section (pages 18 to 21).

The pups' abilities to home orient were assessed every other day between days 4 and 22. "Home orientation" is defined here as the initiation and maintenance of locomotion toward the nest area, when the pup is displaced from the nest within the home cage. More specifically, in the present test a pup was described as displaying home orientation if at the end of a 2 min test it had locomoted to and remained in the home quadrant of the cage.

The mother, litter and food bowl were removed prior to testing and the location of the nest was noted. The clear polycarbonate cage, which housed the female and litter, rested on a piece of manila-colored cardboard with markings which divided the home cage into four equal quadrants. The quadrant of the cage containing the nest was designated the home quadrant and the corner of that quadrant, the home corner. The home cage was not square so that one of the corners adjoining the home corner was nearer than the other. The nearest neighboring corner was called the adjacent corner and its quadrant the adjacent quadrant. The adjacent corner was 28 cm from the home corner. The corner diagonally opposite the home corner was called the diagonal corner and its quadrant the diagonal quadrant. The diagonal corner was approximately 43 cm from the home corner. Pups were tested in the adjacent, home and diagonal corners of the home cage. One cup of the soiled home cage bedding was placed in the home corner and one cup of fresh shavings was placed in each of the other three corners of the cage.

The pup was placed on the cage floor with its head facing away from the test corner towards the center of the cage and the pup was then released. Pup movements during each 2 min test were traced on a facsimile of the home cage and the total number of quadrants crossed was counted. The latency to leave the test quadrant was noted. Finally, the total number of 15 sec intervals during which 5 sec of "no locomotion" occurred was noted. "No locomotion" was defined as the absence of walking and pivoting movements. A pup was tested for 2 min following placement in each of the three corners, with a 30 sec time-out between tests.

There are six possible orders in which a pup could be tested in the three corners. The six combinations were randomly assigned to the six pups within a litter, for a particular day. The order in which the pup was tested in each of the three corners was changed for each day of testing. The order in which the pups were tested was also randomized and changed each day.

Orientation testing of the pups of a particular litter occurred after the mother of the litter had been observed for maternal behavior. Furthermore, orientation testing occurred at the same time each day for a given litter, while observations of all litters within a group were randomly distributed throughout the day.

Results

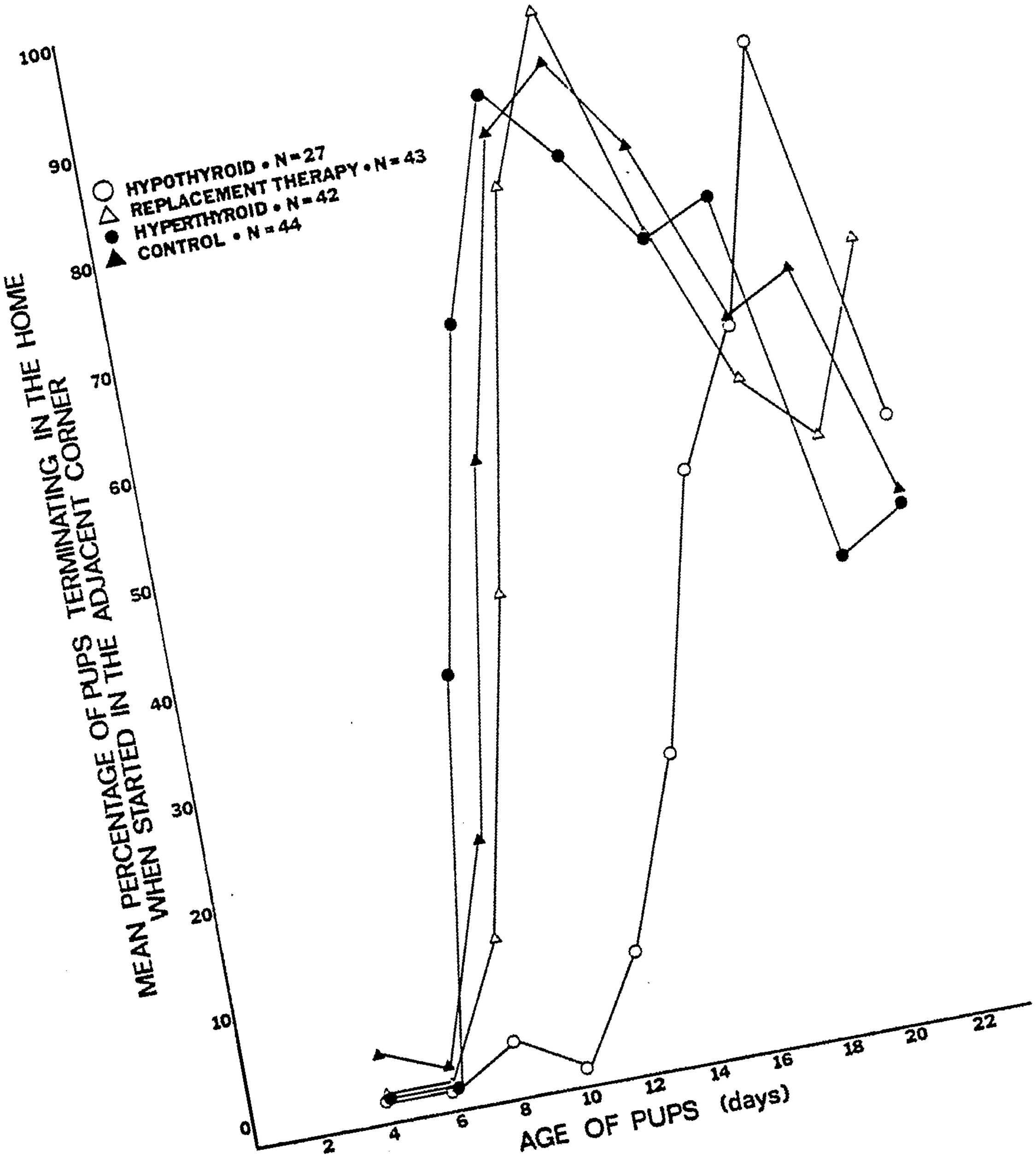
Because of possible litter effects, the litter was considered the unit of analysis in all analyses of pup behavior (Abbey and Howard, 1973). Hence, data for a particular treatment were obtained by averaging pup scores within each litter and then averaging litter scores within the treatment group. Means and standard errors are presented in the Appendix.

In general, hyperthyroid, replacement therapy and control pups behaved quite similarly on the home orientation test, while hypothyroid pups showed a marked delay in the development of home orientation ability.

Percentage of Pups Displaying Homing

Figure 8 shows the mean percentage of pups ending in the home quadrant when started in the adjacent corner, as a function of age. There appears to be little difference among hyperthyroid, control and replacement therapy pups. By days 8 to 10, homing occurred in approximately 50% of the pups. All three types of pup showed a peak of between 80% and 90% ending in the home quadrant between days 12 and 16,

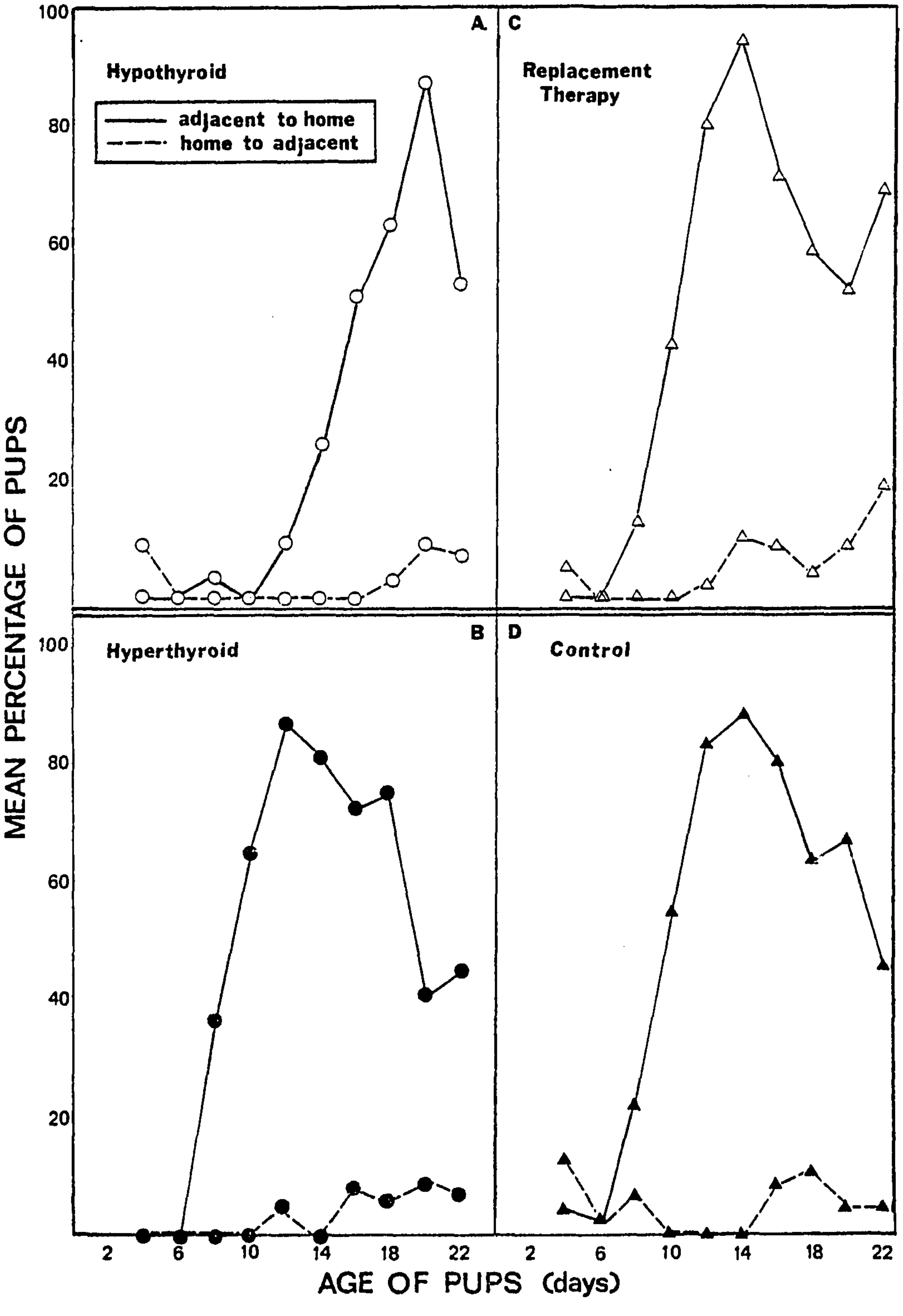
Figure 8. Mean percentage of pups ending in the home quadrant when started in the adjacent corner, as a function of age. Means and standard errors are presented in the Appendix, Table I.


MEAN PERCENTAGE OF PUPS TERMINATING IN THE HOME
WHEN STARTED IN THE ADJACENT CORNER
100
90
80
70
60
50
40
30
20
10
0
○ HYPOTHYROID • N=27
△ REPLACEMENT THERAPY • N=43
● HYPERTHYROID • N=42
▲ CONTROL • N=44
2
4
6
8
10
12
14
16
18
20
22
AGE OF PUPS (days)

after which homing declined to around 50% on day 22. In hypothyroid litters, only 10% of the pups were homing by day 12 and not until day 16 were 50% of the pups ending in the home corner. The peak percentage occurred at day 20, with 87.6% of the pups ending in the home. By day 22, the percentage of pups homing declined to 52.4%. It is interesting to note that the maximum percentage of hypothyroid pups ending in the home quadrant did not differ much from the maximum percentage of other types of pups. The peak was displaced, however, by 4 to 8 days. Variance analysis of these data indicated a significant treatment effect ($\underline{F} = 11.5$, $\underline{df} = 3/28$, $p < 0.001$), a significant age effect ($\underline{F} = 44.77$, $\underline{df} = 9/252$, $p < 0.001$) and a significant Treatment x Age interaction ($\underline{F} = 4.59$, $\underline{df} = 27/252$, $p < 0.001$). Post-hoc comparisons revealed that hypothyroid litters differed from litters in other treatment groups on days 10, 12 and 14 (Scheffé, $p < 0.05$).

For each treatment group, a comparison of the mean percentage of pups ending in the home when started from the adjacent corner can be made with the mean percentage ending in the adjacent quadrant when started from the home. Since the distances are identical, the comparison can be used to measure the relative attractiveness of the nest region to the pups. Figure 9, which compares for each treatment group the percentage of pups moving from the home to the adjacent quadrant and the percentage moving from the adjacent to the home quadrant, clearly shows that very few pups terminated in the adjacent quadrant when started in the home. In

Figure 9. Mean percentage of pups terminating in the home quadrant when started in the adjacent corner is compared to the mean percentage of pups terminating in the adjacent quadrant when started in the home corner, for each of the four treatment groups.

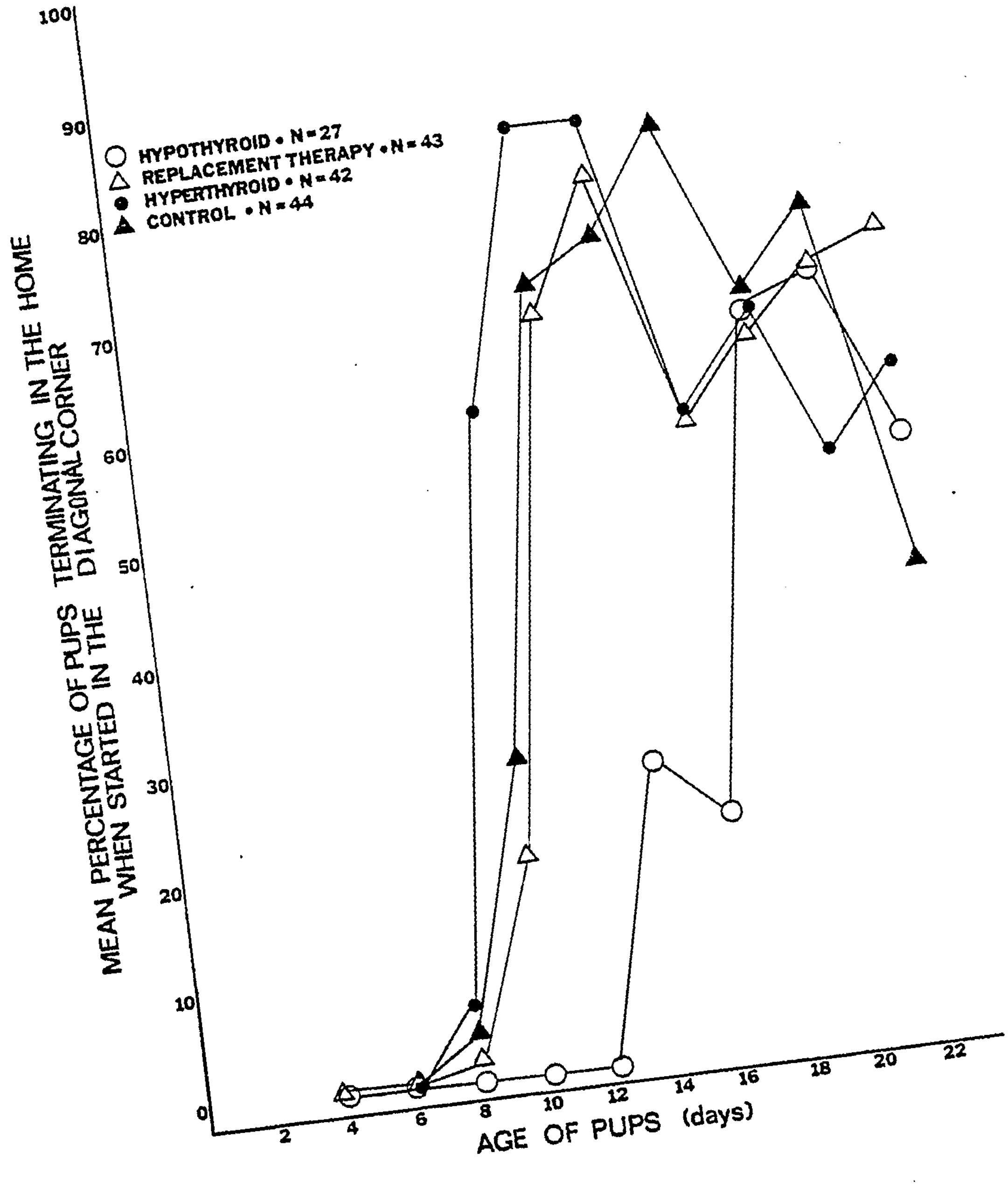

A
Hypothyroid
adjacent to home
home to adjacent
C
Replacement Therapy
B
Hyperthyroid
D
Control
MEAN PERCENTAGE OF PUPS
100
80
60
40
20
AGE OF PUPS (days)
2
6
10
14
18
22

contrast, a large percentage terminated in the home when started from the adjacent corner. This indicates that the home quadrant was strongly preferred by all types of pups.

Similar findings were obtained when pups were started from the diagonal corner, as can be seen in Figure 10. Variance analysis confirms a significant treatment difference ($F = 8.95$, $df = 3/28$, $p < 0.001$) and a significant age effect ($F = 74.33$, $df = 9/252$, $p < 0.001$), as well as a significant interaction between age and treatment ($F = 5.55$, $df = 27/252$, $p < 0.001$). Hypothyroid pups differed from hyperthyroid pups on days 10, 12 and 14; they differed from control pups on days 12, 14 and 16; and from pups receiving replacement therapy on days 12 and 14 (Scheffé, $p < 0.05$). The mean percentage of pups ending in the diagonal quadrant when started from the home corner was compared with the mean percentage ending in the home quadrant when started from the diagonal corner. The findings were similar to those obtained from tests in the adjacent corner. For all treatment groups, the percentage of pups ending home when started in the diagonal corner far exceeded the percentage ending in the diagonal corner when started from the home corner. In other words, the home quadrant was strongly preferred by pups in all groups, even if the development of homing ability was delayed, as in hypothyroid litters.

An analysis of the mean percentage of pups ending in the home quadrant when started from the home corner supports the idea that the home corner was preferred equivalently

Figure 10. Mean percentage of pups terminating in the home quadrant when started in the diagonal corner, as a function of age. Means and standard errors are presented in the Appendix, Table J.


○ HYPOTHYROID • N=27
△ REPLACEMENT THERAPY • N=43
● HYPERTHYROID • N=42
▲ CONTROL • N=44
MEAN PERCENTAGE OF PUPS TERMINATING IN THE HOME DIAGONAL CORNER WHEN STARTED IN THE
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
22
AGE OF PUPS (days)

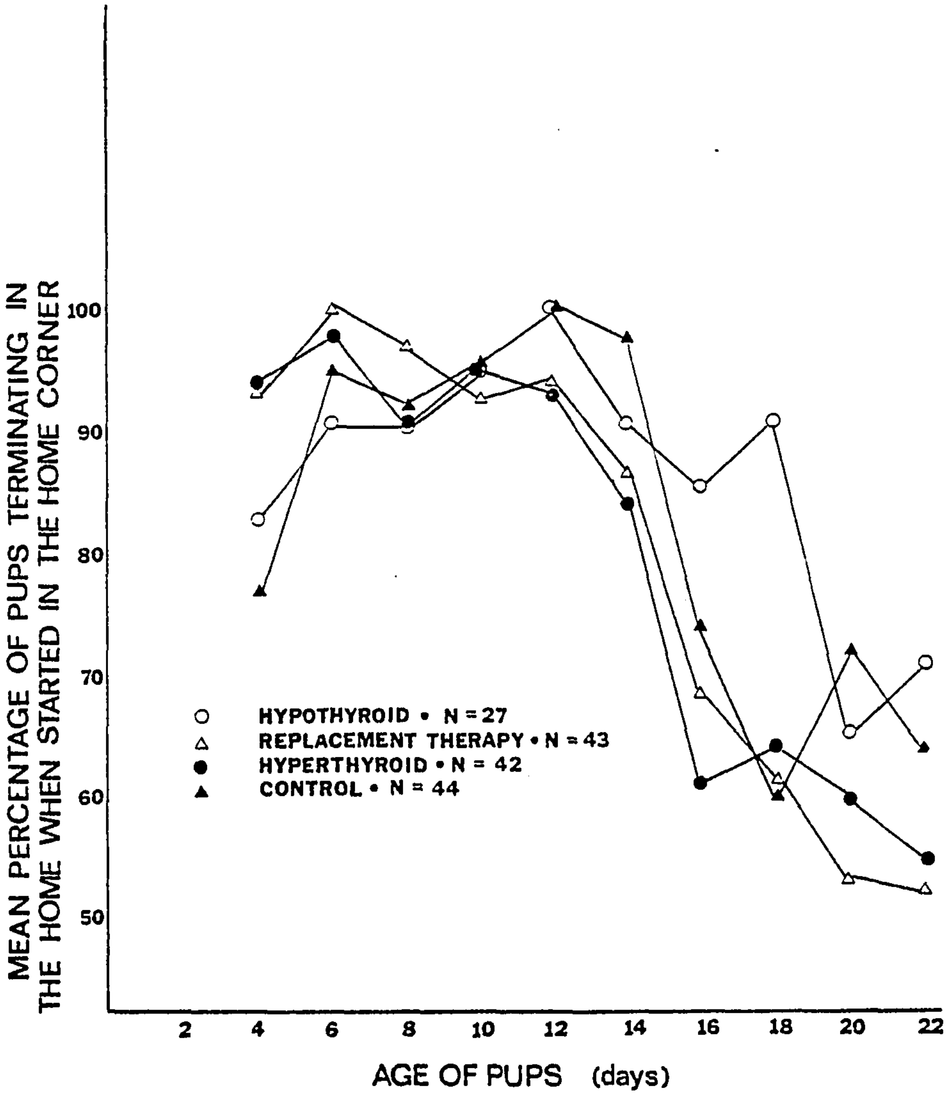
by pups from all groups (see Figure 11). Until day 14, pups in all treatments tended to remain in the home corner or to return to the home before the end of testing. After day 14, hyperthyroid, replacement therapy and control pups showed a decline in the mean percentage of pups terminating in the home. Hypothyroid litters showed a similar decline after day 18. There was no significant treatment effect ($\underline{F} = 0.99$, $\underline{df} = 3/28$, $p = 0.410$) and no Treatment x Age interaction ($\underline{F} = 1.46$, $\underline{df} = 27/252$, $p = 0.071$), but there was a significant age effect ($\underline{F} = 23.77$, $\underline{df} = 9/252$, $p < 0.001$).

The degree of relationship between the modal day of eye opening and the day on which homing declined was assessed across all litters in all treatment groups, in order to assess the hypothesis that eye opening contributes to the decline in homing in rats. A positive relationship existed between day of eye opening and the decline in homing ($\underline{r} = + 0.67$, $\underline{df} = 30$, $p < 0.01$). Since hypothyroidism delays both eye opening and homing, it is likely that the high correlation was obtained because both eye opening and homing were affected by hypothyroidism, and not because eye opening contributes to the decline in homing. When the correlation coefficient was computed excluding the hypothyroid litters, there was no significant correlation between day of eye opening and decline of homing ($r = + 0.34$, $\underline{df} = 23$, $p > 0.05$).

Amount of Locomotor Activity

When the mean number of 15 sec intervals with no locomotor movement was computed for tests starting in the adjacent and diagonal corners, the hypothyroid pups again showed marked

Figure 11. Mean percentage of pups terminating in the home quadrant when started in the home corner, as a function of age. Means and standard errors are presented in the Appendix, Table K.


MEAN PERCENTAGE OF PUPS TERMINATING IN THE HOME WHEN STARTED IN THE HOME CORNER
100
90
80
70
60
50
2
4
6
8
10
12
14
16
18
20
22
AGE OF PUPS (days)
○ HYPOTHYROID • N = 27
△ REPLACEMENT THERAPY • N = 43
● HYPERTHYROID • N = 42
▲ CONTROL • N = 44

differences in behavior in comparison to other pups. Hyperthyroid, replacement therapy and control litters locomoted very little on days 4 and 6. Locomotion increased steadily after day 6 until day 14, when the pups only averaged one 15 sec interval during which 5 sec of "no locomotion" occurred, and activity declined slightly on days 20 and 22. Hypothyroid pups remained relatively quiescent until days 10 to 12, and then showed a gradual decrease in the number of intervals during which no movement occurred. By day 20, there was no difference between these pups and pups in other groups in the mean number of intervals with no locomotion. In tests from the adjacent corner, there was a significant age effect ($\underline{F} = 196.5$, $\underline{df} = 9/252$, $p < 0.001$) and a significant treatment effect ($\underline{F} = 10.87$, $\underline{df} = 3/28$, $p < 0.001$), as well as a significant Age x Treatment interaction ($\underline{F} = 4.35$, $\underline{df} = 27/252$, $p < 0.001$). Hypothyroid pups differed significantly from hyperthyroid and replacement therapy pups on days 12 to 16, and from control pups on days 12 and 14 (Scheffé, $p < 0.05$). The results when pups were tested from the diagonal corner were essentially the same. The treatment effects were highly significant in tests from the diagonal corner ($\underline{F} = 10.69$, $\underline{df} = 3/28$, $p < 0.001$), as were the age effects ($\underline{F} = 229.09$, $\underline{df} = 9/252$, $p < 0.001$) and the Age x Treatment interaction ($\underline{F} = 5.68$, $\underline{df} = 27/252$, $p < 0.001$). Post-hoc comparisons revealed that hypothyroid pups differed from hyperthyroid and control pups on days 10 to 14 and from replacement therapy pups on days 12 and 14 (Scheffé, $p < 0.05$).

When pups were tested from the home corner, however, the hypothyroid pups were found not to differ from pups in other treatments until day 12, considerably later than when tested from the adjacent and diagonal corners. On days 4 to 10, pups in all groups averaged between 6 and 6.5 15 sec intervals (out of a maximum of 8) in which 5 sec of "no locomotion" occurred. There was a gradual decline in the number of intervals with no movement after day 12 for hypothyroid and day 10 for other pups, until by days 20 and 22, pups in all four groups showed no differences. The treatment effect was not significant (_F_ = 1.21, _df_ = 3/28, p = 0.323), but there was a significant effect of age (_F_ = 130.85, _df_ = 9/252, $p < 0.001$) and a significant interaction between age and treatment (_F_ = 3.29, _df_ = 27/252, $p < 0.001$). Post-hoc comparisons revealed that the hypothyroid litters differed from the hyperthyroid litters only on day 12 (Scheffé, $p < 0.05$), but failed to reveal other differences among the treatment groups.

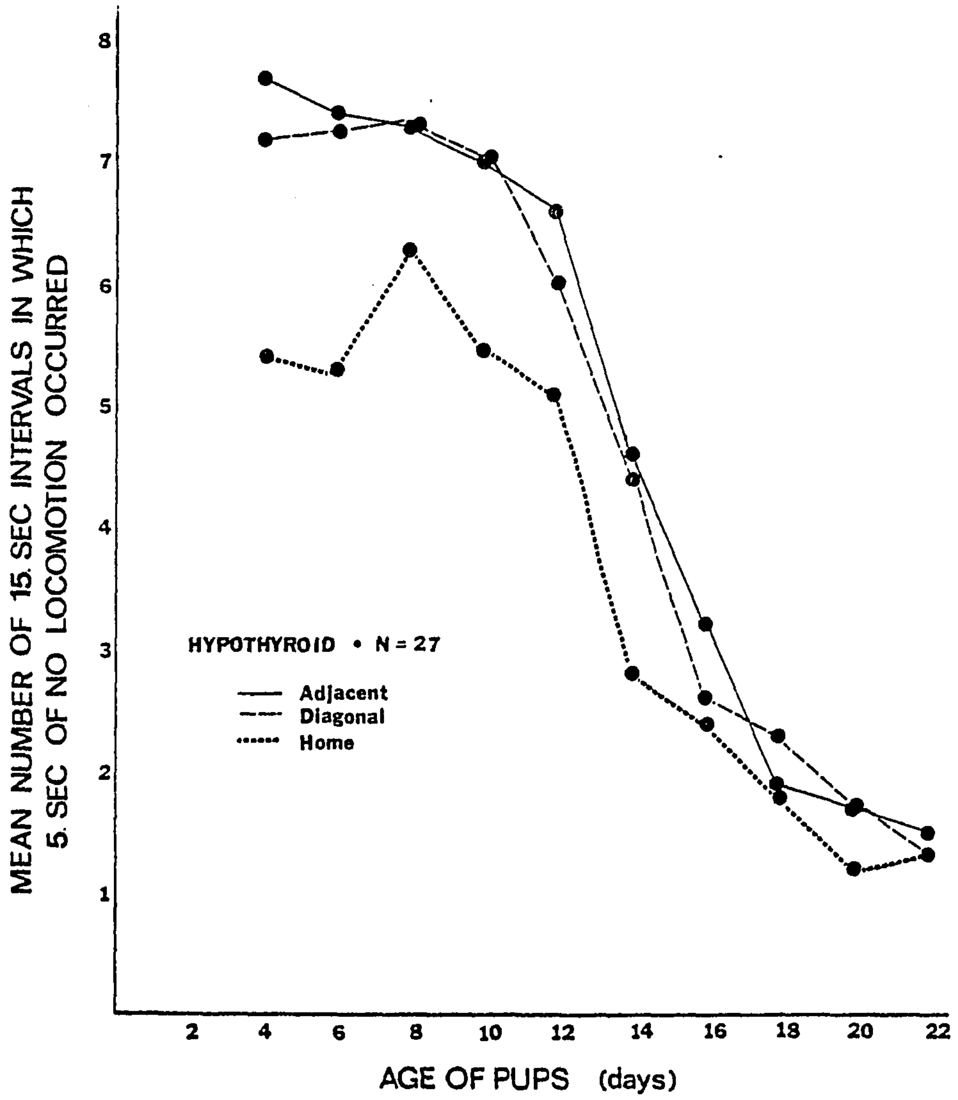
It should be noted that pups may have moved out of the test quadrant at any time during the test. Hence the total number of intervals with "no locomotion" may include intervals which occurred outside of the test quadrant. This may acccount for some of the differences among the treatment groups, since after day 8 there were considerable differences in the percentage of pups showing home orientation.

For all treatments, placement of the pup in the home corner had an activating effect on the pup during the early litter period. This effect appeared to be more marked in the hypothyroid and control pups than in the hyperthyroid pups and in animals receiving replacement therapy. A variance analysis with both test corner and age as repeated factors indicates that there were significant differences in responding, depending on the test corner (_F_ = 21.2, _df_ = 2/56, $p < 0.001$). By day 8, this "activating" effect had disappeared for hyperthyroid, replacement therapy and control pups, but hypothyroid pups continued to show more locomotor activity when placed in the home corner until after day 16 (see Figure 12). The interaction between test corner and treatment was significant (_F_ = 7.10, _df_ = 6/56, $p < 0.001$). The Test Corner x Age interaction was also significant (_F_ = 6.35, _df_ = 18/504, $p < 0.001$), reflecting the fact that the "activating" effect was limited to the early litter period.

Number of Quadrants Crossed

Hypothyroid pups differed from pups in other treatment groups in the mean number of quadrants crossed during the 2 min test. When pups were started in the adjacent corner, there was very little movement by pups in any treatment until day 10. After day 10, control, replacement therapy and hyperthyroid pups showed a gradual increase in the number of quadrants crossed, peaking at days 16 to 18 with approximately 20 crossings per test. Hypothyroid animals showed a much slower increase in the number of quadrants crossed, peaking at day 22 with an average of 11.9 crossings. Treatment thus had a significant effect on the mean number of quadrants crossed (_F_ = 22.15, _df_ = 3/28, $p < 0.001$), as did age (_F_ = 158.01, _df_ = 9/252, $p < 0.001$). Furthermore, there was a

Figure 12. Mean number of 15 sec intervals in which 5 sec of "no locomotion" occurred, for the hypothyroid litters (n = 7 litters, 27 pups).


MEAN NUMBER OF 15. SEC INTERVALS IN WHICH 5. SEC OF NO LOCOMOTION OCCURRED
8
7
6
5
4
3
2
1
HYPOTHYROID • N = 27
Adjacent
Diagonal
Home
2
4
6
8
10
12
14
16
18
20
22
AGE OF PUPS (days)

significant interaction between age and treatment (F = 8.28, df = 27/252, $p < 0.001$), reflecting the increasing differences among treatment groups with age. Scheffé tests revealed that the hypothyroid pups differed from hyperthyroid and replacement therapy pups on days 14 to 20 and from control pups on days 16 to 20 ($p < 0.05$).

Data from tests starting in the diagonal corner or the home corner yield very similar findings. In tests from the diagonal corner, there was a significant effect of treatment on the number of quadrants crossed (F = 26.57, df = 3/28, $p < 0.001$), as well as a significant age effect (F = 157.50, df = 9/252, $p < 0.001$) and a significant Age x Treatment interaction (F = 8.37, df = 27/252, $p < 0.001$). As was the case in tests from the adjacent corner, the hypothyroid pups differed from the hyperthyroid pups on days 14 to 20; from the replacement therapy pups on days 14 to 22; and from control pups on days 16 to 20 (Scheffé, $p < 0.05$). In tests from the home corner, the treatment effect was significant (F = 15.79, df = 3/28, $p < 0.001$), as was the age effect (F = 149.31, df = 9/252, $p < 0.001$) and the Age x Treatment interaction (F = 8.86, df = 27/252, $p < 0.001$). Pair-wise comparisons revealed that the hypothyroid pups differed significantly from hyperthyroid and control pups on days 16 and 18, and from replacement therapy pups on days 14 to 20 ($p < 0.05$).

Latency to Leave the Test Quadrant

When the mean latency to leave the test quadrant was computed, the hypothyroid pups were again markedly different from the pups in other treatment groups. Figure 13 a shows the mean latency to leave the adjacent quadrant as a function of age; a maximum latency of 120 sec was assigned to pups which never left the quadrant. There was little difference among treatments on days 4 and 6, but by day 8 control, hyperthyroid and replacement therapy pups were leaving more rapidly than hypothyroid pups. Indeed, animals from hypothyroid litters did not show a decline in latency until after day 12, and they were always slower to leave the quadrant than other pups. The treatment effect was highly significant ($\underline{F} = 40.92$, $\underline{df} = 3/28$, $p < 0.001$), as was the age effect ($\underline{F} = 275.68$, $\underline{df} = 9/252$, $p < 0.001$) and the Treatment x Age interaction ($\underline{F} = 7.44$, $\underline{df} = 27/252$, $p < 0.001$). Post-hoc comparisons indicated that hypothyroid pups showed significantly slower latencies on days 10 to 16 when compared with hyperthyroid and control pups and on days 10 to 18 when compared with pups receiving thyroxine replacement therapy (Scheffé, $p < 0.05$). It is possible that the hypothyroid pups differed from litters in other treatment groups because they were less likely to leave the test quadrant, and not because they left more slowly. However, when the latency data were computed for only those pups who left the starting quadrant, a longer latency by the hypothyroid pups was still apparent (see Figure 13 b). Results from the diagonal corner were essentially the same.

When the latency to leave the home quadrant was computed for all pups, as shown in Figure 14 a, there appears to be no difference among treatments until day 10. After this age, hypothyroid pups again seemed to be consistently

Figure 13. Mean latency to leave the adjacent quadrant as a function of pup age. (A) All animals are included and a maximum of 120 sec is assigned to pups which never leave the test quadrant. Means and standard errors are presented in the Appendix, Table R. (B) Data included only those animals which do leave the test quadrant during the 2 min test.

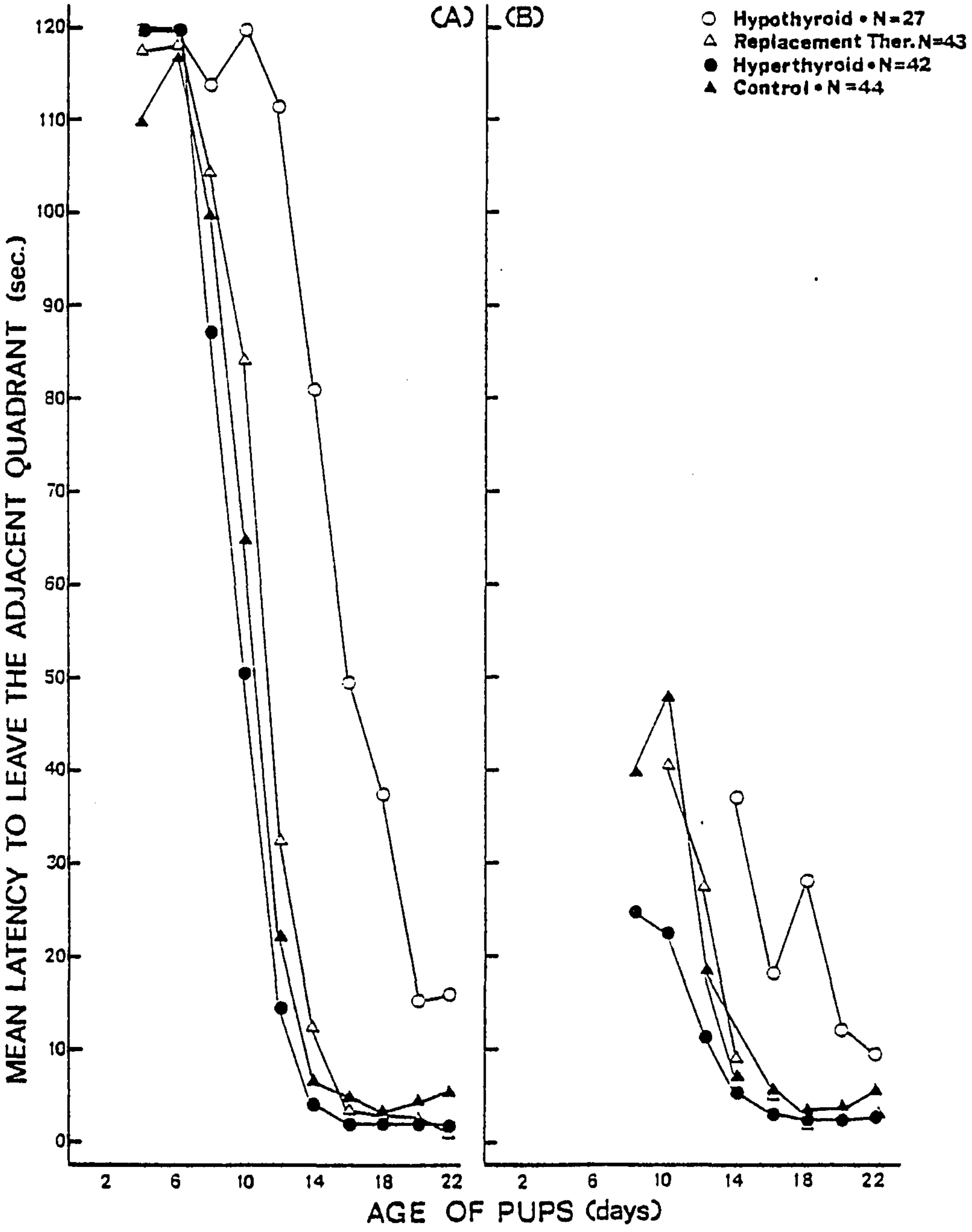
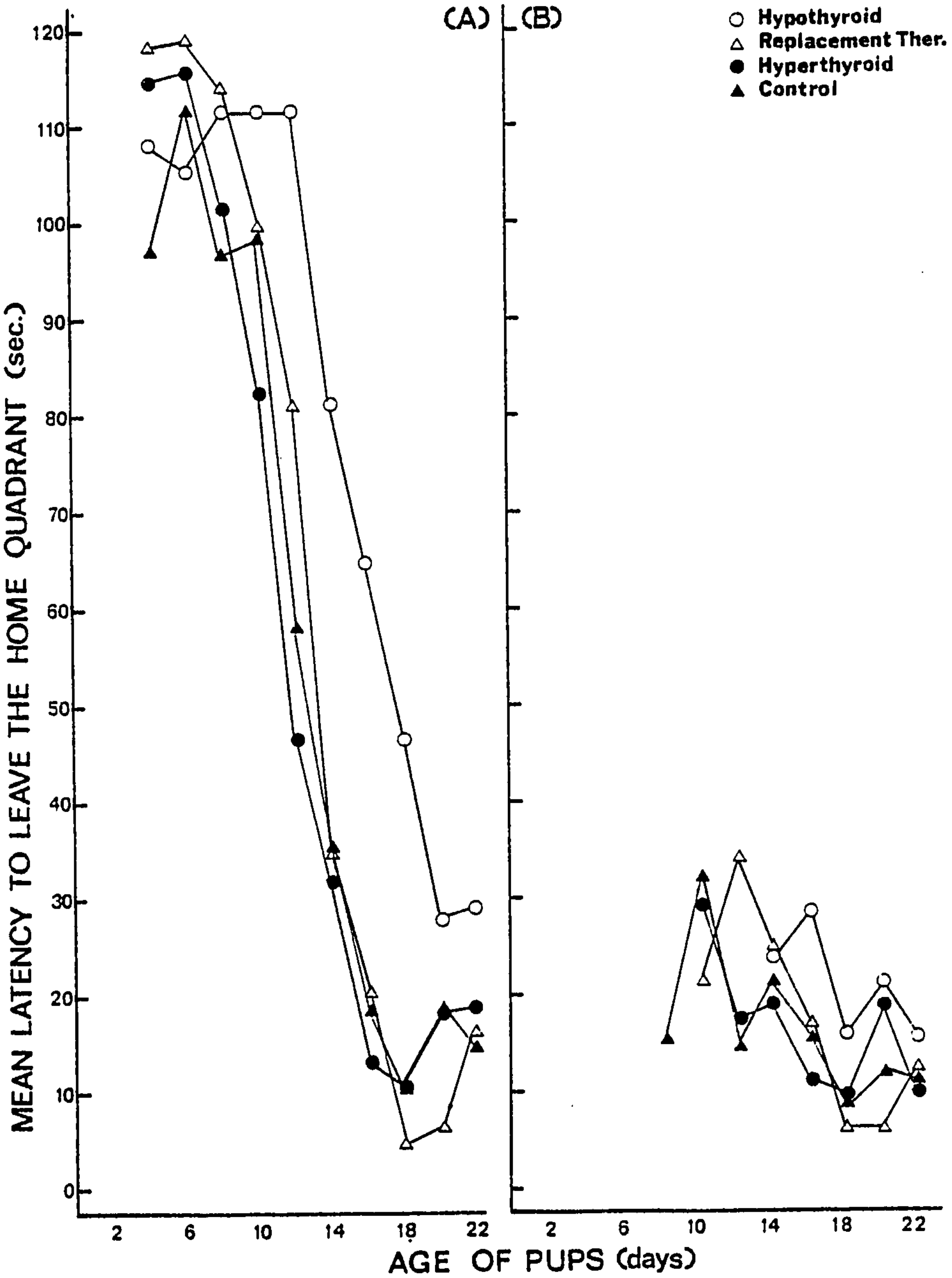

(A)
(B)
○ Hypothyroid • N=27
△ Replacement Ther. N=43
● Hyperthyroid • N=42
▲ Control • N=44
MEAN LATENCY TO LEAVE THE ADJACENT QUADRANT (sec.)
0
10
20
30
40
50
60
70
80
90
100
110
120
AGE OF PUPS (days)
2
6
10
14
18
22
2
6
10
14
18
22

Figure 14. Mean latency to leave the home quadrant as a function of pup age. (A) All animals are included and a maximum of 120 sec is assigned to pups which never leave the test quadrant. Means and standard errors are presented in the Appendix, Table T. (B) Data included only those animals which do leave the home quadrant during the 2 min test.


(A)
(B)
○ Hypothyroid
△ Replacement Ther.
● Hyperthyroid
▲ Control
MEAN LATENCY TO LEAVE THE HOME QUADRANT (sec.)
0
10
20
30
40
50
60
70
80
90
100
110
120
AGE OF PUPS (days)
2
6
10
14
18
22
2
6
10
14
18
22

slower to leave the home corner. The difference among treatments was found to be significant (F = 8.87, df = 3/28, $p < 0.001$). The Age x Treatment interaction was also significant, reflecting the fact that the differences among groups appeared only after days 8 to 10 (F = 4.61, df = 27/252, $p < 0.001$). The hypothyroid pups differed significantly from hyperthyroid pups on days 12 and 16 and from control pups on day 12 (Scheffé, $p < 0.05$), but did not differ significantly from replacement therapy pups. If the latencies were computed to include only those pups which did leave the home corner during the test, the differences among treatments were eliminated (F = 2.64, df = 3/28, p = 0.069), as can be seen in Figure 14 b.

Summary of Results

1) Hypothyroid pups were delayed in the development of homing, compared to pups in other treatment groups.

2) The peak percentage of hypothyroid animals showing homing was not different from the peak percentage of control pups, even though the emergence of homing was delayed in these pups.

3) Pups in all treatment groups showed a greater amount of activity when placed in the home corner than when placed in other corners of the cage. This effect was most marked early in the litter period.

4) Hypothyroid pups crossed fewer quadrants and showed longer latencies to leave the test quadrant than control pups.

5) There were no differences in homing among hyperthyroid pups, control pups and pups receiving replacement therapy.

Discussion

Home orientation is a complex response requiring a rather detailed analysis of environmental information, and an integration of this sensory input with a motor output, i.e. locomotion toward a particular area. Nevertheless, the home orientation response emerged apparently intact in hypothyroid pups, despite the fact that it developed several days later than in controls.

Pups in all four of the treatment groups clearly responded to the home as a preferred quadrant (see Figures 8, 9 and 10), although hypothyroid pups did not show normal homing until several days after the animals in the other treatment groups. It is interesting to note that the same percentage of hypothyroid pups showed homing as pups in other treatments. In fact, the curve for the development of homing in hypothyroid animals appears to be simply displaced along the abscissa.

The hypothyroid rats showed a deficit which was clearly distinguishable from the deficit in homing observed in malnourished rat pups (Fleischer and Turkewitz, in press). Malnourished pups, i.e. rats reared in artificially large litters, showed consistently lower levels of homing at all ages, unlike the hypothyroid pups, which eventually reached control levels of homing. Homing was delayed in malnourished pups, but not quite as much as in hypothyroid animals. It thus seems likely that the homing deficit observed in young hypothyroid rats is not mediated via malnourishment, but is a more direct effect of hormone insufficiency.

There was a significant relationship between the day of eye opening and the day on which homing declined. However, this was most likely due to the fact that both eye opening and homing were delayed by a common causal factor, hypothyroidism. When hypothyroid litters were eliminated from the analysis, the correlation between day of eye opening and the decline in homing was not significant. This suggests that eye opening is not causally related to the decline in homing.

Hypothyroid animals were considerably less active than the other pups tested, and this might account in part for the observed deficit in homing shown by hypothyroid pups. Close examination of the data suggests that the differences in activity may not be sufficient to explain the deficit in homing shown by hypothyroid animals. Hyperthyroid, control and replacement therapy pups only began to shown an increase in the mean number of squares crossed by day 10, at which time a large percentage of these pups were displaying home orientation. Hypothyroid pups showed a similar increase in the number of quadrants crossed by day 12, but they failed to show a peak in homing until day 20. Hypothyroidism thus appears to delay somewhat independently both development of locomotor activity and the development of homing. It seems unlikely then that the differences in the development of homing are due entirely to differences in motor development.

Pups in all treatment groups were able to discriminate between the home quadrant and other quadrants, even at very young ages. In fact, placement in the home quadrant appeared to have an activating effect on the behavior of the very young rat (see Figure 12). When 4 to 6 day old pups were placed in the home corner, they showed fewer intervals with 5 sec of no locomotion than when placed in either the adjacent or diagonal corners. In control, hyperthyroid and replacement therapy litters, this differential responsiveness disappeared after day 8, while hypothyroid pups continued to show more movement in the home quadrant until day 16. Tobach, Rouger and Schneirla (1967) and Tobach (1977) also report more activity in young rat pups when they are placed on home cage shavings than fresh shavings.

The differential responding in the home quadrant can also be seen if the latency to leave the test quadrant is examined. When pups were tested from either the adjacent or diagonal corner, hypothyroid pups showed longer latencies to leave than control pups (see Figure 13 a). Even if the data were adjusted to include only those pups which did leave the quadrant, as in Figure 13 b, there were still significant differences in latency to leave. This suggests that hypothyroid pups did move more slowly. If the latency data from tests in the home corner are examined, it seems as if hypothyroid pups also left the home more slowly than controls (see Figure 14 a). However, if the data include only those animals which do leave the home, as in Figure 14 b, the latency differences among the treatment groups disappear. Hypothyroid pups who leave the home corner do so as rapidly as controls, but in general hypothyroid animals are less likely to leave the home than other pups. This finding lends further support to the idea that hypothyroid pups could discriminate between the home quadrant and other quadrants of the home cage.

The sensory basis for the differential responding was not assessed in the current study of home orientation. However, Tobach, Rouger and Schneirla (1967) reported that increases in activity when pups were placed on home cage shavings were abolished following removal of the olfactory bulbs. It seems likely then that in the current study, the differences in movement reflected differences in the odor of home cage and fresh shavings, although differential responding on the basis of thermal or tactile cues might be involved.

The evidence suggests that the deficits in homing are not attributable to total anosmia in early development. This does not preclude the possibility that hypothyoid pups are less sensitive to olfactory gradients and that this lack of sensitivity, along with the retardation in motor development shown by these pups, may explain the observed deficits in homing.

With respect to the question raised in the introduction, it seems that an integrated behavioral response such as homing is affected by perinatal hormone insufficiency. The emergence of the homing response was delayed from 4 to 8 days in young hypothyroid rat pups, but the response appeared relatively normal once it emerged. Furthermore, the peak percentage of hypothyroid rat pups homing was equivalent to the peak percentage of control and hyperthyroid pups. The deficit in homing shown by hypothyroid pups was quite different from that shown by malnourished rats, suggesting that the effects of hypothyroidism on homing are not mediated exclusively by the stunting of the pup. Perinatal hormone excess did not accelerate the emergence of the home orientation response and generally these pups were indistinguishable from control animals on the basis of this response pattern, even though eye opening was accelerated by two days in hyperthyroid pups.

IV. DEVELOPMENT OF OLFACTORY AND THERMAL RESPONSIVENESS

Introduction

Young hypothyroid rats showed a considerable delay in the development of the home orientation response, and questions may be raised as to the nature of the underlying deficit. There are a variety of sensory cues that could be used as the basis for orientation and it is possible that a delay in the development of one or more of the sensory systems responsive to these cues would result in a delay in the development of the orientation response. Proximal cues, such as thermal, olfactory or tactile cues, are likely to be most important in guiding the orientation response shown by previsual and preaudial rats.

Responsiveness to differences in thermal stimulation may be in part responsible for the development of the homing response, since a thermal gradient is established around the mother and litter in the nest area. Leonard (1974) reports that hamster pups are extremely sensitive to thermal gradients. Hamsters less than eight days old moved rapidly and reliably toward a heat source, when tested on a thermal gradient of less than a degree across the body surface. Leonard suggests that the movement of the hamster pup is related to the difference between the ambient temperature and the body temperature of the pup and may be responsible for the pup's burying behavior in the nest. It seems likely that such sensitive responding could also account for the homing of pups when they are displaced from the nest region. Responsiveness to thermal stimulation was examined in infant rats by Fowler and Kellogg (1975). Animals younger than 5 days failed to move toward a warm compartment, but by 6 to 7 days the pups spent more time in the warm compartment than the cool compartment. The pups also showed marked differences in behavior when placed in the two compartments, with pups 6 to 10 days old showing much less movement within the warm compartment. It is clear that rat pups are able to respond differentially to thermal cues, but Fowler and Kellogg failed to present evidence that the pups showed strongly directed orienting responses similar to those shown by hamster pups. It is possible, however, that the differential pattern of movement in warm and cool environments would be sufficient to allow a kinetic orientation to thermal stimuli, so that thermal cues could serve in home orientation by infant rats.

Several studies suggest that olfactory cues may be especially important in homing in rats. Tobach, Rouger and Schneirla (1967) studied the role of olfaction in the discrimination of home and nonhome cages by neonatal rat pups. They tested pups in home and clean cage pans, either empty or filled with home sawdust or fresh sawdust. In tests in which bedding was present in the cage pan, they found the greatest amount of activity when pups were tested in the home pan with home sawdust and the least amount of activity in animals tested in a fresh pan with fresh sawdust. Removal of the olfactory bulbs abolished all differences in activity on the different substrates, strongly suggesting that olfactory cues were important in the differential responsiveness. Gregory and Pfaff (1971) placed pups at the junction of soiled home cage shavings and fresh shavings and measured the percentage of pups moving into either the soiled or fresh shavings. They found that an increasing percentage of pups moved into the home cage shavings after 7 days, the level of discrimination becoming significant at 12 days. Tobach (1977) reports that even one day old pups discriminate between home cage and fresh shavings by showing more activity on the soiled shavings.

Not only can pups discriminate between home cage shavings and fresh shavings, but they also can demonstrate a marked responsiveness to maternal odors. Schapiro and Salas (1970) found that by day 2 the scent of an air current bearing odor from a lactating female inhibited ongoing behavior in 56% of the animals tested, while 40% showed a decline with no maternal odor. By day 8, this difference increased substantially; 76% showed a response to maternal odor, as compared to 14% without maternal odor. Finally, Leon and Moltz (1971, 1972) have demonstrated that young rats are able to discriminate between the odor of a lactating female and the odor of a nonlactating female. In conclusion, pups clearly discriminate between the odor of home cage shavings and the odor of fresh shavings, and also between the odor of lactating females and the odor of nonlactating females, making it likely that olfactory cues are important in home orientation.

There are good reasons to believe that thermal and olfactory responsiveness might be affected by early thyroid hormone manipulation. Hamburgh (1968) reports that hypothyroid rats show a considerable delay in the development of homeothermy and he suggests that animals made hypothyroid during early development may never become completely homeothermic. It seems quite possible that the delay in the development of homeothermy may affect the responsiveness of the young hypothyroid rat to thermal stimulation. Olfactory functioning is also likely to be affected by early hormone manipulation. Since the olfactory bulb of rats undergoes much of its differentiation postnatally (Altman and Das, 1966; Salas, Guzman-Flores and Schapiro, 1969), rendering it susceptible to thyroid hormone insufficiency or excess, it seems possible that the deficit in homing observed in hypothyroid rats might be due to a deficit in the development of olfactory responsiveness. Thus, for the final portion of the investigation, an additional set of litters was tested for responsiveness on a thermal and an olfactory gradient, in an attempt to clarify the underlying basis for the observed differences in home orientation.

Method

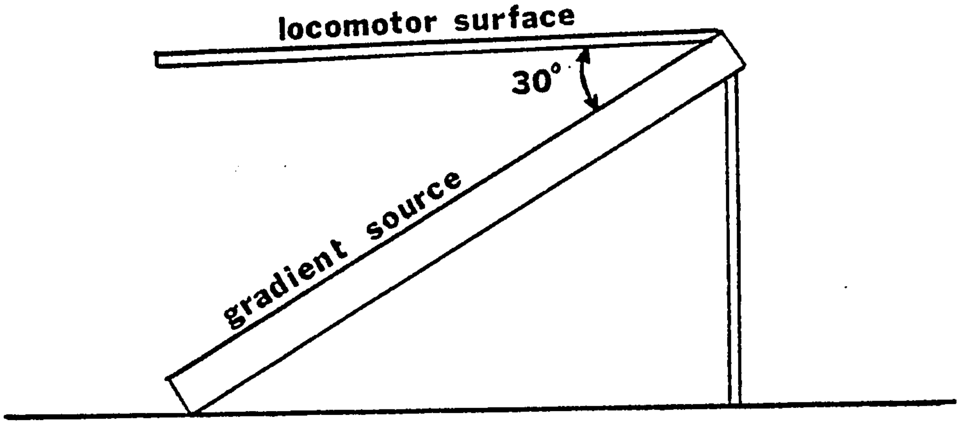
Subjects

The offspring from twenty Charles River CD strain rats were used for this portion of the investigation. Five litters were assigned to each of the four treatment groups. Data were not used from pups which showed a continuing weight loss during the testing resulting in death before day 22. To minimize the differences in maternal care received by litters of varying sizes, only data from litters of more than three pups were used. Two replications were made to obtain a sufficient number of pups in each of the treatment groups. The resulting data were obtained from 18 hypothyroid rats, 27 hyperthyroid rats, 30 control rats and 28 rats receiving thyroxine replacement therapy.

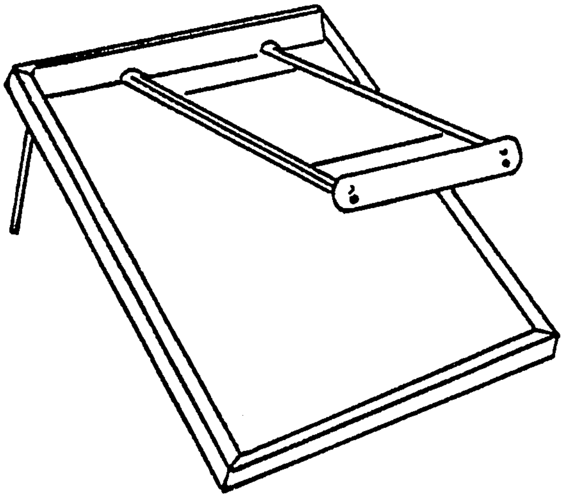
Apparatus

The thermal and olfactory gradients were established using the apparatus shown in Figure 15. Two identical apparatuses were used, one for the thermal gradient and one for the olfactory gradient. The gradient source, either a heating pad or a container filled with soiled home cage shavings, was placed on a wooden base which measured 36 x 43 cm. The gradient source was at a 30° angle to the surface on which the pups moved. As a result, different points along the locomotor surface were increasingly closer to or further from the gradient source. The locomotor surface was constructed by stretching a piece of plastic mesh screening between two wooden dowels. The dowels, measuring 1 cm in diameter and separated from each other by 15 cm, rose at a 30° angle from one side of the base of the apparatus, thus forming a 38 cm long platform on which the pups could walk. The platform was approximately 18 to 20 cm above the gradient source at the furthest point. Two 6 cm start zones were marked with colored thread on opposite ends of the locomotor surface. A second pair of dowels, measuring 0.5 cm in diameter, were placed over the first pair to prevent the younger pups from wandering off the edge of the platform. Finally, a piece of wood was slipped over the ends of the dowels to keep the platform

Figure 15. Apparatus used to assess the pups' responsiveness on olfactory and thermal gradients.



A. Side view of the gradient apparatus



B. Olfactory and thermal gradient apparatus rigid. The entire structure was raised at a 30° angle so that the locomotor surface was level.

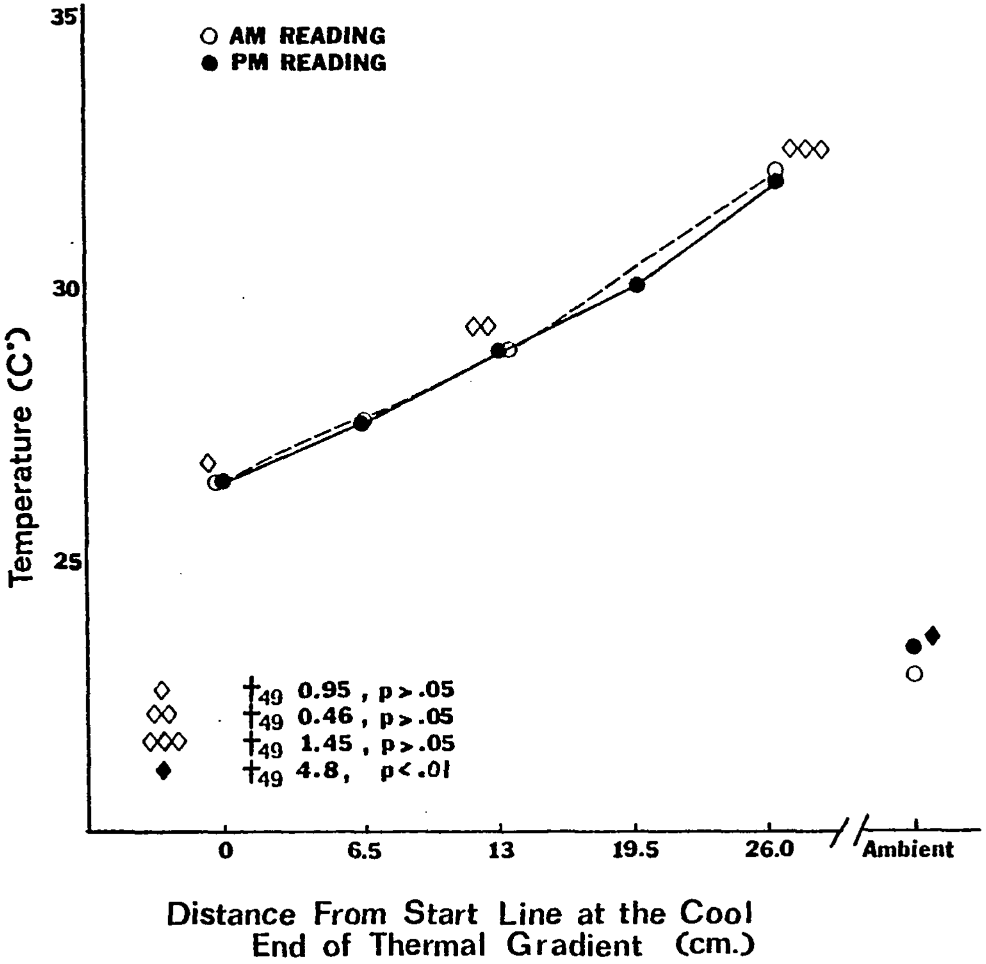
The gradient source was placed on the wooden base and extended into the two start zones. The heat was generated from an Oster 3 heat, wet-proof heating pad (Model 780-01, 120 volt, 55 watt; Oster Corporation, Milwaukee, Wisconsin). A second Oster heating pad was used to keep pups warm during testing. The thermal gradient was measured prior to testing and at the end of each day using three Taylor air thermometers. The gradient was linear between the two start lines (see Figure 16) and did not change significantly from the start to the end of testing. The gradient changed at the rate of 0.23 C° per cm. Ambient temperature was measured with a Taylor recording thermometer, which was located 1.5 m from the gradient apparatus. There was a small, but significant, rise in ambient temperature from a mean of 22.9°C prior to testing to a mean of 23.3°C at the end of testing ($\underline{t} = 4.8$, $\underline{df} = 49$, $p < 0.01$), but the small difference in ambient temperature was not sufficient to alter the temperature gradient.

The olfactory gradient was established by placing 2 cups of soiled shavings into a 17 x 32 x 1 cm plastic container. To prevent the shavings from sliding when the apparatus was tilted, several strips of cardboard 0.5 cm high rose from the bottom of the container.

Procedure

Litters were housed in 27 x 36 cm metal cages. They were maintained as described on pages 18 to 21. The methods for inducing hypo- and hyperthyroidism and for providing

Figure 16. Temperature (in degrees centigrade) along the locomotor surface of the thermal gradient. The temperature was measured at 0, 6.5, 13.0, 19.5 and 26.0 cm from the cool end of the thermal gradient.


35
30
25
Temperature (C°)
○ AM READING
● PM READING
◇ t_{49} 0.95 , p > .05
◇◇ t_{49} 0.46 , p > .05
◇◇◇ t_{49} 1.45 , p > .05
◆ t_{49} 4.8, p < .01
0
6.5
13
19.5
26.0
Ambient
Distance From Start Line at the Cool End of Thermal Gradient (cm.)

replacement therapy were identical to those described in the first series of experiments (pages 18 to 21).

The litters were observed every other day, starting on day 4 and continuing until just before the modal day of eye opening for each treatment group. Testing thus continued through day 12 for replacement therapy and hyperthyroid litters; day 14 for control pups; and day 16 for hypothyroid pups. Testing was discontinued at eye opening since it was apparent in pilot work that young rats generally failed to remain on the locomotor surface for the duration of testing after eye opening had occurred.

Gradient tests for a particular litter were made at approximately the same time each day, while observations of litters within a treatment group were randomly distributed throughout the day.

The heating pads were turned on approximately 20 to 30 min prior to testing the first litter. The pad serving as the heat source for the thermal gradient was set on "high" and the three Taylor thermometers were placed 0, 13 and 26 cm from the start zone at the cool end of the thermal gradient. Testing began when a stable temperature gradient was established. The second heating pad, which served to keep pups warm during testing, was set on the "medium" temperature setting. Two cups of soiled home cage shavings from the litter being tested were uniformly scattered in the container used to generate the olfactory gradient.

The mother was removed from the home cage and was placed in a holding cage for the duration of testing, while the pups were kept in a small plastic cage containing clean shavings. Each of the pups was tested starting in both the cool and warm ends of the thermal gradient, and the weak and strong ends of the olfactory gradient. The order in which the four tests were given was random, as was the order in which the pups within a litter were tested.

For 3 min prior to each test, the pup was placed in a plastic cup on the heating pad. The young rat was tested for 1 min on one of the gradients, after which it was given a 3 min timeout on the heating pad before the next 1 min test. Two pups were tested simultaneously. While one pup was being tested the other received a 3 min timeout on the heating pad.

The animal being tested was placed on the locomotor surface perpendicular to the gradient. Approximately 3 sec later, two stopwatches were started simultaneously. One stopwatch recorded the total elapsed test time, while the second recorded the cumulative amount of time which the rat spent in the start zone. The number of 5 sec intervals during which no movement occurred was recorded. "No movement" was defined as the complete absence of both locomotor and nonlocomotor movement, with the exception of occasional twitches. Finally, the distance from the start line to the pup's location at the end of 1 min was noted.

Results

The procedure in testing for the development of responsiveness on the olfactory and thermal gradients involved observations on animals until just before the modal day of eye opening. Graphical presentation of the data is made with all data points indicated, so that curves for the hypothyroid litters extend to day 16, for control litters to day 14 and for hyperthyroid and replacement therapy litters to day 12. However, variance analysis of the data includes only those days when litters in all treatment groups were tested, i.e. days 4 to 12. When appropriate, one-way analyses of variance were made to compare the performances of pups in different treatment groups on the last day of testing for each treatment. Post-hoc comparisons were made using Dunnett's tests. Means and standard errors are given in the Appendix.

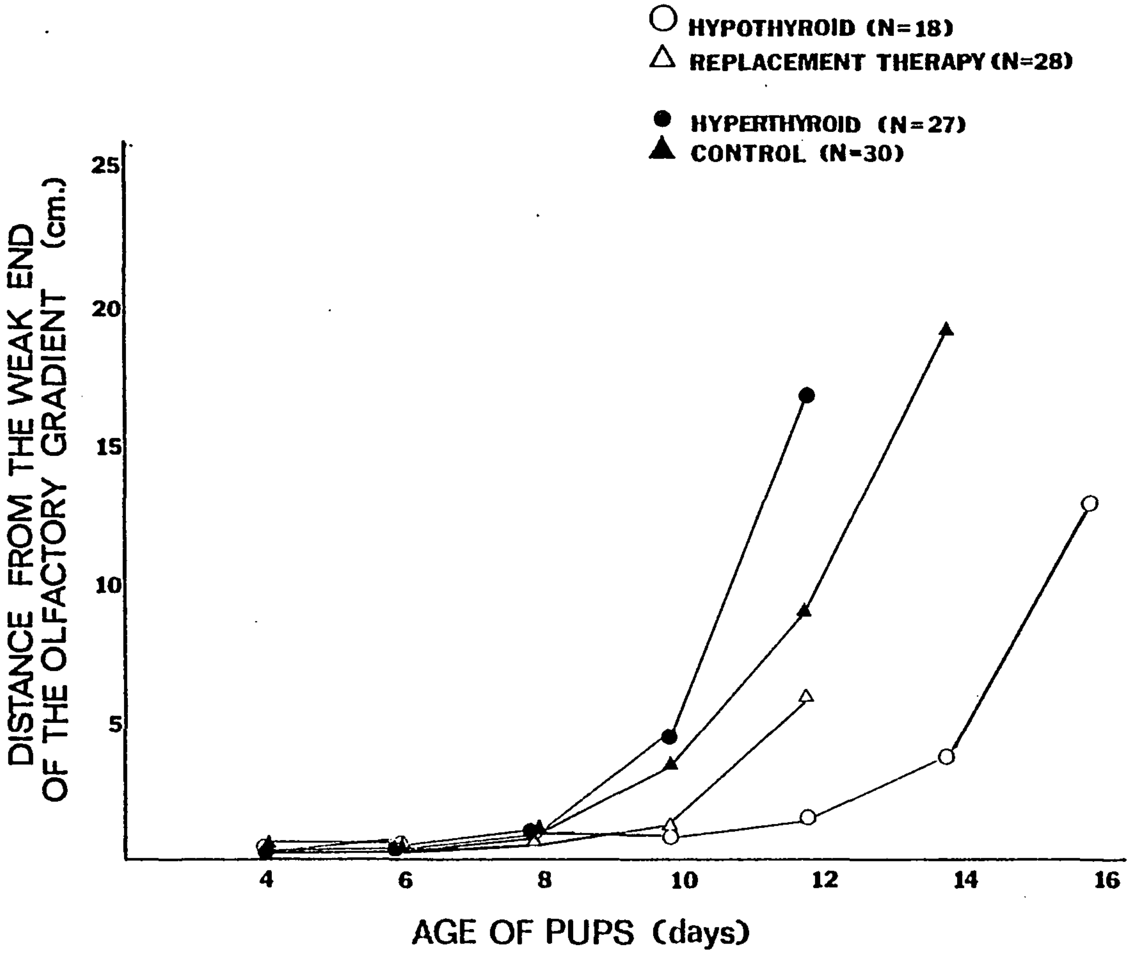
For each pup, the number of 5 sec blocks during which no movement occurred while the pup was in the 6 cm start zone was totalled and then multiplied by five to obtain the total amount of time (in blocks of 5 sec) in which the pup was not moving. This figure was then transformed into a percentage of the total time spent in the start zone. The resulting datum represents the percentage of the total time in the start during which the pup was not moving (in blocks of 5 sec).

Olfactory Gradient

Distance from the Weak End

The litters in the four treatment groups showed considerable differences in the distance of their terminal location from the start line when they were tested from the weak end of the olfactory gradient, as can be seen in Figure 17. On days 4 to 8, pups showed very little movement from the start

Figure 17. Mean distance (in cm) from the weak end of the olfactory gradient, as a function of age. Means and standard errors are presented in the Appendix, Table U.


○ HYPOTHYROID (N=18)
△ REPLACEMENT THERAPY (N=28)
● HYPERTHYROID (N=27)
▲ CONTROL (N=30)
DISTANCE FROM THE WEAK END OF THE OLFACTORY GRADIENT (cm.)
25
20
15
10
5
4
6
8
10
12
14
16
AGE OF PUPS (days)

zone, but after day 8 hyperthyroid and control pups began to move outside of the start zone. Replacement therapy pups began to move after day 10, and hypothyroid pups did not show much movement from the start zone until day 14. Variance analysis of the data show that the treatment effect was significant ($\underline{F} = 14.06$, $\underline{df} = 3/16$, $p < 0.001$), as was the age effect ($\underline{F} = 53.18$, $\underline{df} = 4/64$, $p < 0.001$) and the Treatment x Age interaction ($\underline{F} = 8.86$, $\underline{df} = 12/64$, $p < 0.001$). Dunnett's tests revealed that on day 12, the control pups moved significantly further than hypothyroid pups and hyperthyroid pups moved significantly further than the controls ($p < 0.05$). Replacement therapy pups did not differ from the control pups at any age. A one-way analysis of variance comparing the distances on the final day of testing for each of the treatment groups indicates a significant difference among the treatments ($\underline{F} = 5.46$, $\underline{df} = 3/16$, $p < 0.01$), probably due to the failure of replacement therapy pups to show orientation along the gradient before testing was terminated. It seems likely that the difference stems from the fact that testing was terminated two days before the modal day of eye opening in these animals, while testing for hyperthyroid and control litters terminated the day before the modal day of eye opening.

Activity in the Weak End

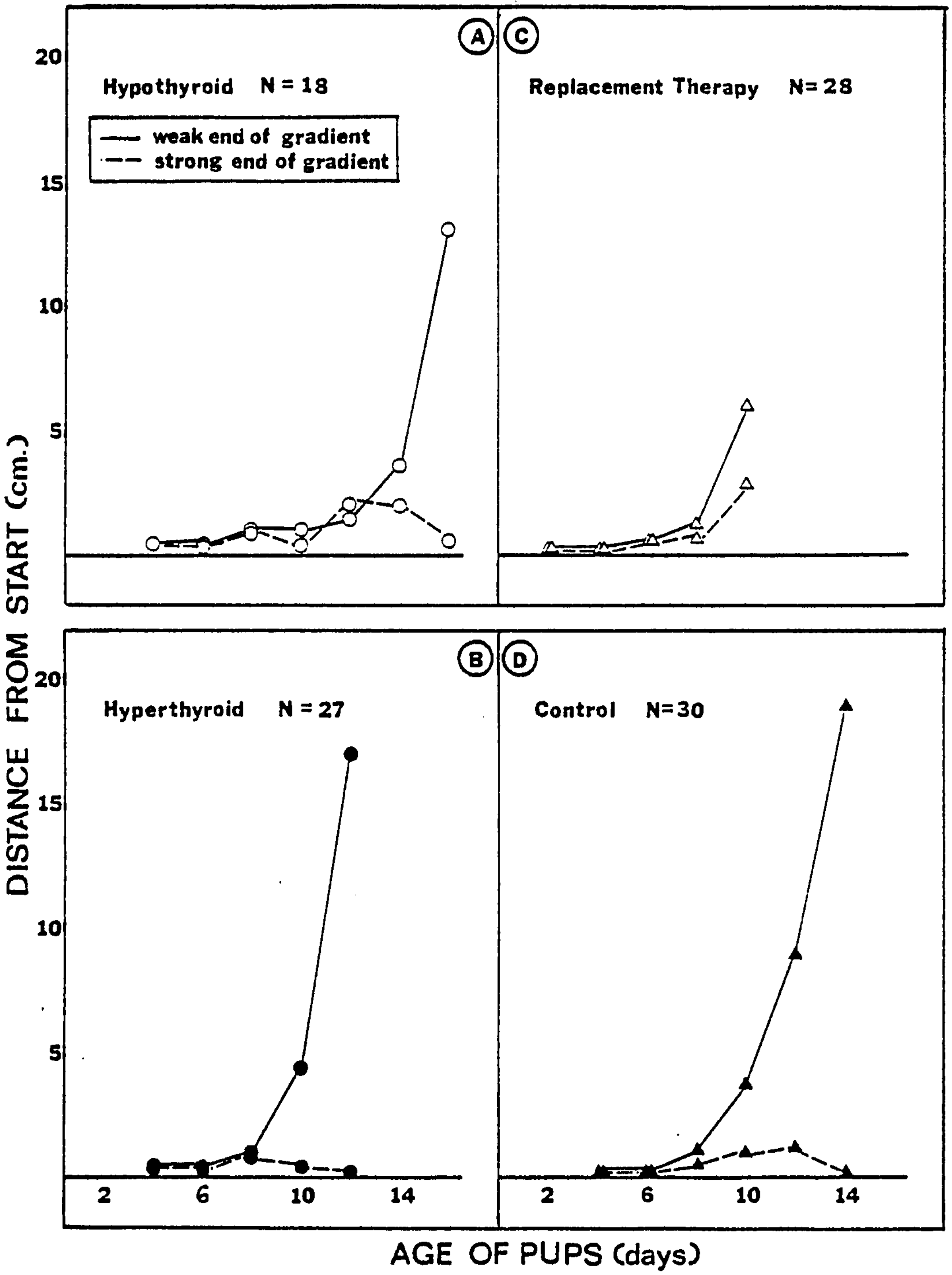
No differences were found among treatments in the mean percentage of time during which no movement occurred, when pups were started in the weak end of the olfactory gradient ($\underline{F} = 1.32$, $\underline{df} = 3/16$, $p = 0.302$). There was no significant interaction between treatment and age (*F* = 0.50, *df* = 12/64, p = 0.905), but there was a significant age effect (*F* = 69.82, *df* = 4/64, $p < 0.001$), with pups spending between 60% and 80% of the time in the start not moving on day 4 and between 0% and 5% on day 12.

Distance from the Strong End

When pups were started from the strong end of the olfactory gradient, there were no differences among treatments in the distance moved (*F* = 0.64, *df* = 3/16, p = 0.599), as well as no interaction between age and treatment (*F* = 1.50, *df* = 12/64, p = 0.146). There was a significant effect of age, however (*F* = 6.25, *df* = 4/64, $p < 0.001$). On days 4 to 10, pups in all treatment groups rarely moved outside of the start zone. By day 12, there was some movement outside the start zone, but this was minimal.

Figure 18 compares the terminal distance when pups were started from the weak end of the olfactory gradient with the distance when pups were started from the strong end of the olfactory gradient. It is clear that when older pups were placed in the weak end of the gradient, they tended to move toward the strong end, and when placed in the strong end, they tended to remain there. Animals which moved from the weak end of the gradient to the strong end generally did so directly, with little movement back toward the start zone. In contrast, those animals which left the strong end of the gradient generally returned immediately.

Figure 18. Mean distance from the weak end of the olfactory gradient compared to the mean distance from the strong end of the olfactory gradient, for each of the four treatment groups. Means and standard errors are presented in the Appendix, Table U.


Ⓐ
Ⓒ
Ⓑ
Ⓓ
Hypothyroid N = 18
weak end of gradient
strong end of gradient
Replacement Therapy N= 28
Hyperthyroid N = 27
Control N=30
20
15
10
5
DISTANCE FROM START (cm.)
2
6
10
14
AGE OF PUPS (days)

Activity in the Strong End

As can be seen in Figure 19, there are significant differences in the mean percentage of time in the start zone in which the pup was not moving (F = 3.77, df = 3/16, $p = 0.03$). There was little difference among treatments until after day 8, at which time hypothyroid pups appeared to spend a slightly larger percentage of time not moving. The age effect was significant (F = 79.60, df = 4/64, $p < 0.001$), but there was no interaction between age and treatment (F = 1.41, df = 12/64, $p = 0.184$).

When the mean percentage of time not moving when pups were in the weak end of the olfactory gradient is compared to the mean percentage of time in the strong end of the gradient, as in Figure 20, it is clear that significant differences exist in the responses of the young rats at the two ends of the gradient (F = 49.3, df = 1/16, $p < 0.001$). Surprisingly, in light of the "activating" effect of being placed in the home cage shavings during the orientation test, pups showed a higher percentage of time not moving when placed in the strong end of the olfactory gradient. The tests at the two ends of the gradient also showed a significant interaction with treatment (F = 3.99, df = 3/16, $p = 0.027$), in that hypothyroid pups showed the greatest differences in activity in the two tests. Finally, there was no significant interaction between pup age and test (F = 2.01, df = 4/64, $p = 0.103$).

Figure 19. Mean percentage of time spent in the start zone at the strong end of the olfactory gradient, during which no movement occurred. Means and standard errors are presented in the Appendix, Table V.

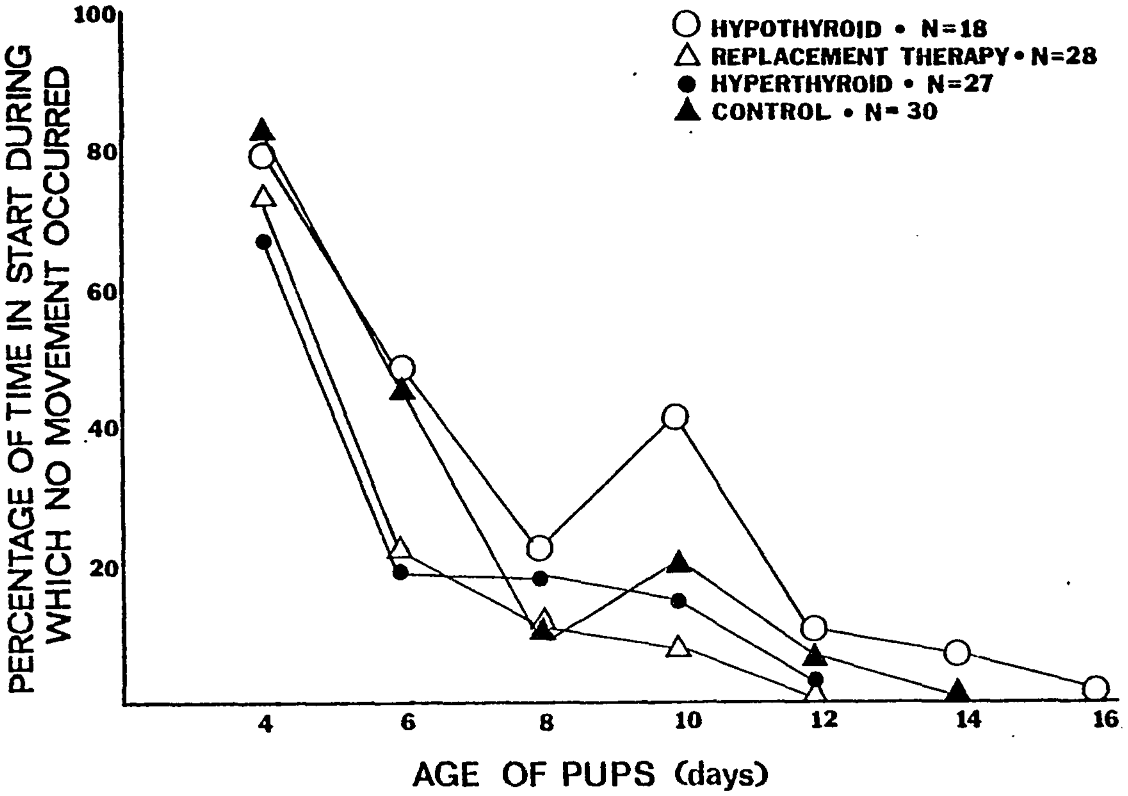
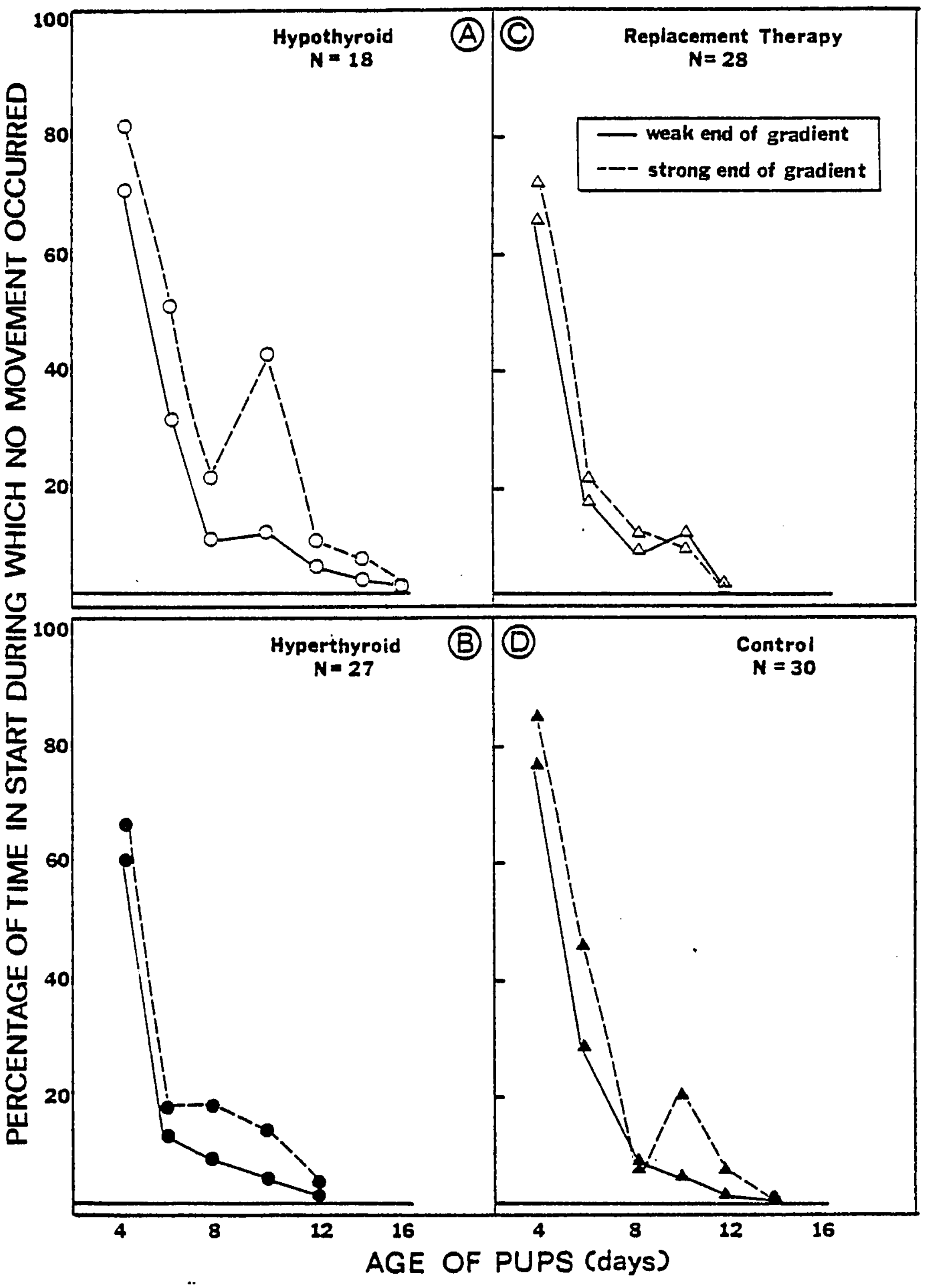

HYPOTHYROID • N=18
REPLACEMENT THERAPY • N=28
HYPERTHYROID • N=27
CONTROL • N= 30
PERCENTAGE OF TIME IN START DURING WHICH NO MOVEMENT OCCURRED
100
80
60
40
20
4
6
8
10
12
14
16
AGE OF PUPS (days)

Figure 20. Mean percentage of time spent in the start zone during which no movement occurred, when pups were in the weak end of the olfactory gradient, is compared to the mean percentage of time when pups were in the strong end of gradient, for each of the four treatment groups. Means and standard errors are presented in the Appendix, Table V.


PERCENTAGE OF TIME IN START DURING WHICH NO MOVEMENT OCCURRED
Hypothyroid
N = 18
Ⓐ
Ⓒ
Replacement Therapy
N= 28
weak end of gradient
strong end of gradient
Hyperthyroid
N= 27
Ⓑ
Ⓓ
Control
N = 30
100
80
60
40
20
4
8
12
16
AGE OF PUPS (days)

Olfactory Gradient Control Procedure

Purpose

In order to test the possibility that differences in responsiveness to the olfactory stimulus were due to differences in the timing of secretion of odorous substances by the female (e.g. the maternal "pheromone" described by Leon and Moltz, 1971, 1972), rather than differences in pup capabilities, some pups were tested on an olfactory gradient using a standard olfactory source for animals in all treatment groups.

Method

Day 14 bedding from control litters in the first replication was frozen and used to test responsiveness of the infant rats during the second replication. Day 14 shavings were used since Leon and Moltz (1972) found that day 14 lactating females were extremely attractive to young rats. On day 10, 2 cups of shavings were thawed and pups from the second replication were tested from the weak end of an olfactory gradient generated by the shavings. Day 10 was chosen since control pups of this age are mobile and somewhat responsive to home cage shavings in the present test situation. Thus, on day 10 all pups were given five tests in random order, including a test with day 14 control shavings. In order to determine whether frozen shavings had retained the ability to attract day 14 pups, the control animals were retested with the frozen shavings when they were 14 days old.

If differences in responsiveness on the olfactory gradient were due to differences in the onset of "pheromone" secretion, one would expect day 10 pups from all conditions to respond to the 14 day shavings in the same way. However, if differences in responsiveness reflected differences in the development of the sensory or sensory-motor capabilities of the pups, then one would expect differences in the responsiveness to day 14 shavings among the four treatment groups.

Results

When day 10 pups were tested using soiled shavings from day 14 control litters, it becomes clear that changes in the odor of the shavings with age were not solely responsible for the pattern of responding obtained from the pups. As can be seen in Figure 21, the day 10 pups tended to respond to day 14 shavings as they responded to their own home cage shavings. The frozen shavings retained the ability to attract pups, however, since day 14 control pups responded to the frozen shavings as they responded to their own home cage shavings. The number of litters tested in this way was admittedly small, but in light of the findings, it seems unlikely that changes in the odor of the shavings are solely responsible for the changes in the pups' responses toward the shavings.

Thermal Gradient

Distance from the Cool End

The young rats in the four treatment groups showed substantial differences in the responses shown on the thermal gradient. There were significant treatment differences in the terminal distance from the start zone when animals were tested from the cool end of the thermal gradient ($\underline{F} = 10.79$, $\underline{df} = 3/16$, $p < 0.001$). As can be seen in Figure 22, there

Figure 21. Mean distance from the weak end of the olfactory gradient generated using day 14 control shavings is compared to the mean distance moved on a gradient using the pups' own home cage shavings. Error bars indicate the standard error.

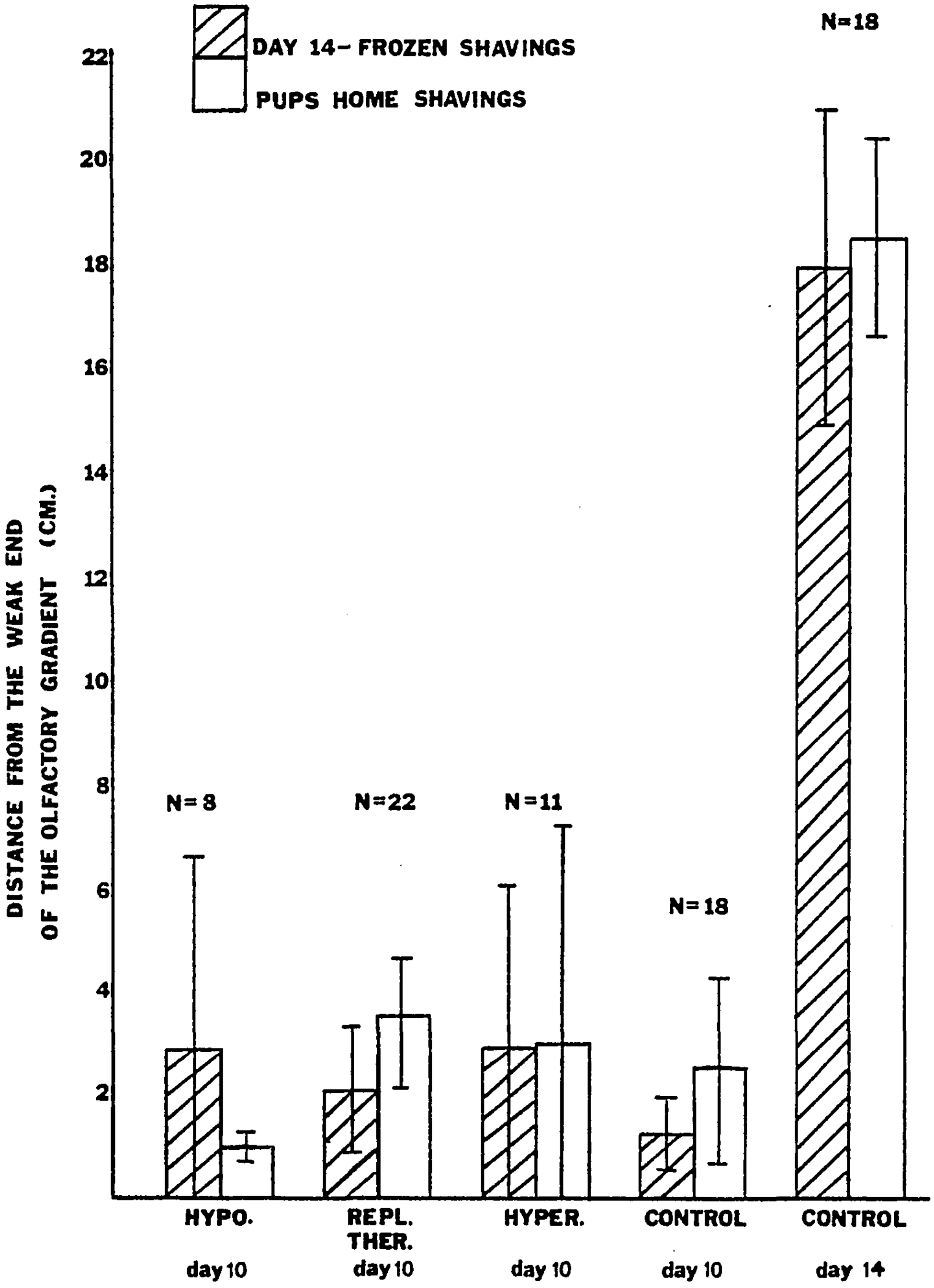
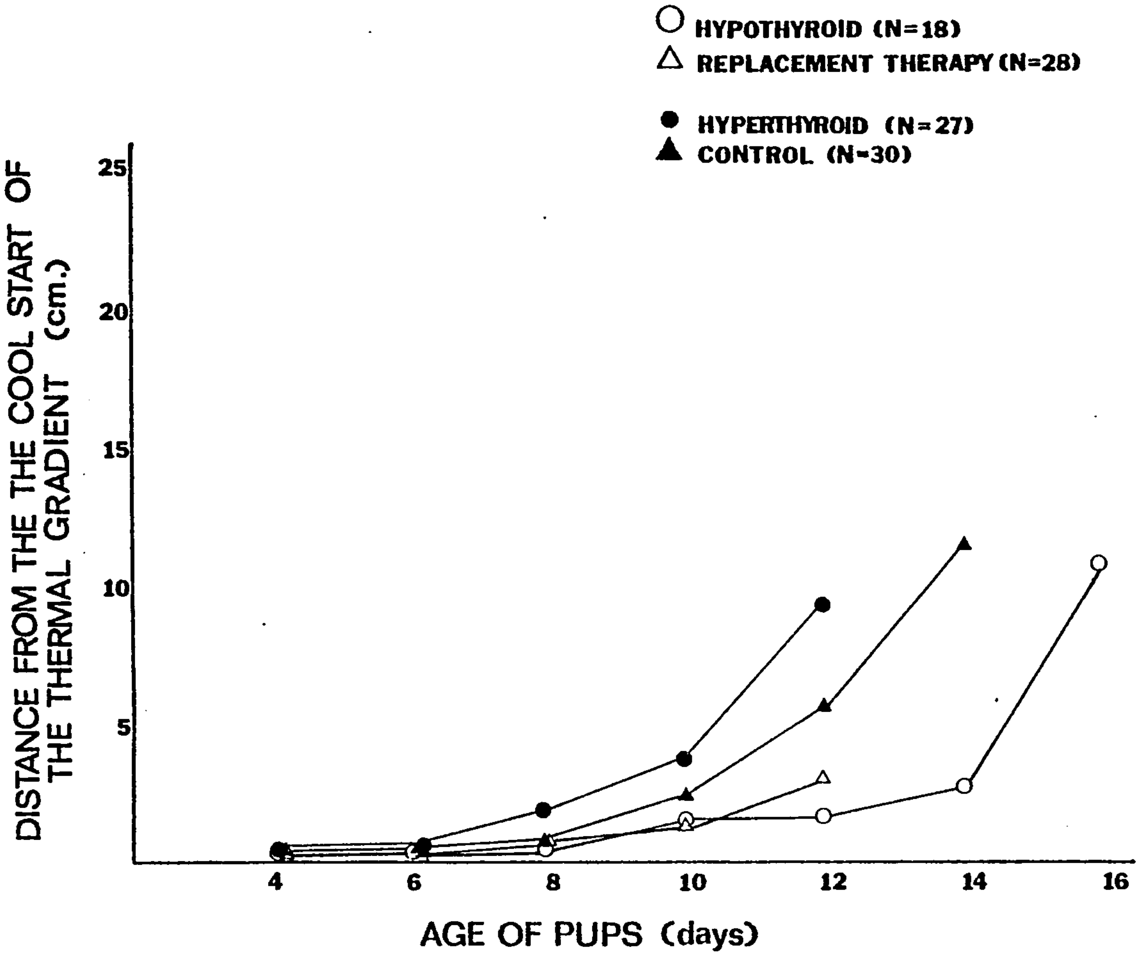

DAY 14- FROZEN SHAVINGS
PUPS HOME SHAVINGS
DISTANCE FROM THE WEAK END OF THE OLFACTORY GRADIENT (CM.)
22
20
18
16
14
12
10
8
6
4
2
N= 8
N=22
N=11
N= 18
N=18
HYPO.
day 10
REPL. THER.
day 10
HYPER.
day 10
CONTROL
day 10
CONTROL
day 14

Figure 22. Mean distance from the cool end of the thermal gradient, as a function of age. Means and standard errors are presented in the Appendix, Table W.


HYPOTHYROID (N=18)
REPLACEMENT THERAPY (N=28)
HYPERTHYROID (N=27)
CONTROL (N=30)
DISTANCE FROM THE THE COOL START OF THE THERMAL GRADIENT (cm.)
25
20
15
10
5
4
6
8
10
12
14
16
AGE OF PUPS (days)

was very little movement by pups on days 4 to 8, but after day 8, pups terminated at increasing distances from the start zone. The age effect was significant ($\underline{F} = 62.04$, $\underline{df} = 4/64$, $p < 0.001$), as was the Age x Treatment interaction ($\underline{F} = 7.25$, $\underline{df} = 12/64$, $p < 0.001$). The control pups moved further than the hypothyroid and replacement therapy pups on day 12, while hyperthyroid litters moved further than controls (Dunnett, $p < 0.05$). A comparison of the terminal distance on the last day of testing for each treatment group indicates that there were no significant differences among treatments ($\underline{F} = 1.94$, $\underline{df} = 3/16$, $p > 0.05$).

For the four treatment groups, the terminal distance when started from the weak end of the olfactory gradient was compared to the terminal distance when started from the cool end of the thermal gradient. The differences in distance on the last day of testing for hypothyroid litters was not significant ($\underline{t} = 0.40$, $\underline{df} = 4$, $p > 0.05$), but the differences were significant for hyperthyroid litters ($\underline{t} = 2.68$, $\underline{df} = 4$, $p < 0.05$), for control litters ($\underline{t} = 4.65$, $\underline{df} = 4$, $p < 0.005$) and for litters receiving replacement therapy ($\underline{t} = 2.97$, $\underline{df} = 4$, $p < 0.025$). Thus, hyperthyroid, replacement therapy and control animals moved significantly further when tested on the olfactory gradient than when tested on the thermal gradient.

Activity in the Cool End

There were no significant differences among treatment conditions in the mean percentage of time during which no movement occurred in the cool end of the thermal gradient

($F = 1.88$, $df = 3/16$, $p = 0.174$) and no significant interaction between treatment and age ($F = 0.95$, $df = 12/64$, $p = 0.506$). There was a significant age effect ($F = 74.55$, $df = 4/64$, $p < 0.001$), with the pups showing a rapid decline in the percentage of time with no movement after day 14.

Distance from the Warm End

The differences among treatments in the terminal distance when pups were started from the warm end of the gradient were significant ($F = 3.19$, $df = 3/16$, $p = 0.052$). There was no interaction effect between treatment and age ($F = 1.2$, $df = 12/64$, $p = 0.306$), but again there was a significant age effect ($F = 11.13$, $df = 4/64$, $p < 0.001$).

Unlike the case with the olfactory gradient, when the terminal distance from the start zone when pups were started from the warm end of the gradient was compared to the terminal distance when pups were started from the cool end, it is clear that there were no differences in the responding, as there were at the two ends of the olfactory gradient. Litters made hyperthyroid tended to move slightly further when started from the cool end of the thermal gradient on day 12 ($t = 2.95$, $df = 4$, $p < 0.05$), but the differences in distance were not significant for hypothyroid litters ($t = 1.36$, $df = 4$, $p > 0.05$), replacement therapy litters ($t = 0.95$, $df = 4$, $p > 0.05$), or control litters ($t = 1.30$, $df = 4$, $p > 0.05$) on the last day on which these treatment groups were tested. There was no evidence that pups moved to a "preferred" temperature along the gradient.

Activity at the Warm End

As Figure 23 shows, animals from hypothyroid litters spent significantly more time not moving in the warm end of the thermal gradient than pups in other treatment groups ($\underline{F} = 14.29$, $\underline{df} = 3/16$, $p < 0.001$). The Age x Treatment interaction was significant ($\underline{F} = 3.07$, $\underline{df} = 12/64$, $p = 0.002$), reflecting the fact that hypothyroid litters differed from other pups only after day 6. Dunnett's tests revealed that the hypothyroid litters differed from the control litters on days 8, 10 and 12 ($p < 0.05$). The age effect was significant ($\underline{F} = 21.99$, $\underline{df} = 4/64$, $p < 0.001$), with the pups in all treatment groups showing a decline in activity with age.

If the percentage of time not moving is compared for tests at the warm and cool ends of the thermal gradient, as in Figure 24, it is clear that pups were differentiating between the two ends of the gradient. Hypothyroid pups showed considerably more movement when placed in the cool end of the gradient than the warm end. Pups in other conditions showed a similar, but less marked, difference in responsiveness.

Summary of Results

1) Hypothyroid animals showed a delay and hyperthyroid animals an acceleration in the development of orientation along the olfactory gradient.

2) Pups in all treatment groups showed more movement at the weak end of the olfactory gradient than at the strong end of the gradient.

3) None of the treatment groups showed directed orientation along the thermal gradient.

Figure 23. Mean percentage of time spent in the start zone at the warm end of the thermal gradient, during which no movement occurred. Means and standard errors are presented in the Appendix, Table X.

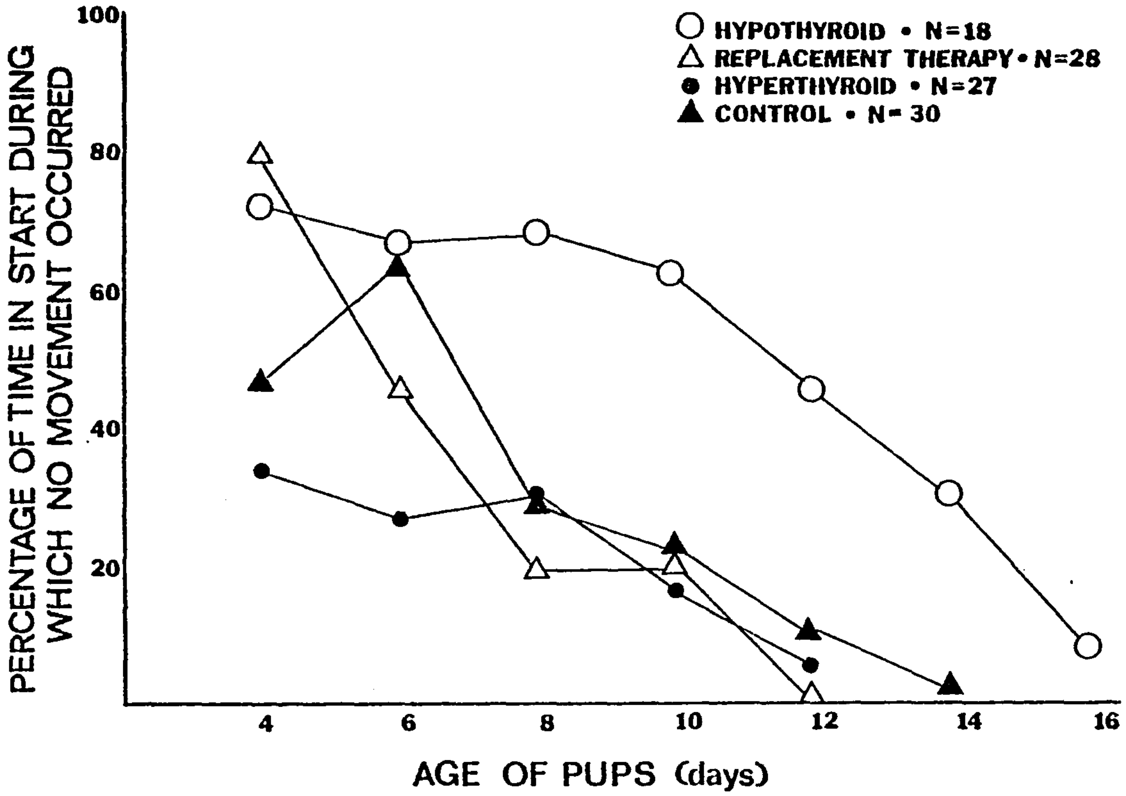
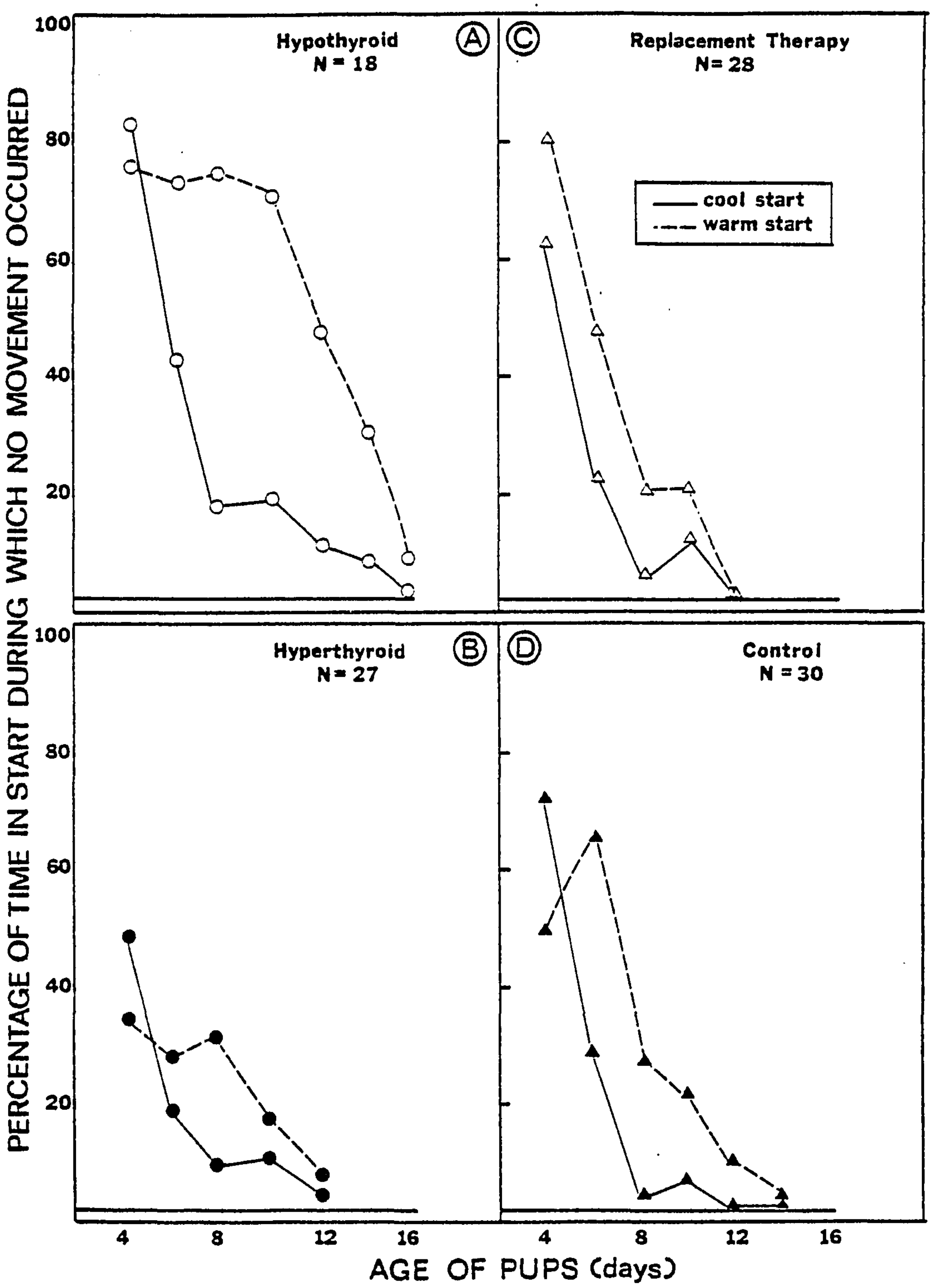

○ HYPOTHYROID • N=18
△ REPLACEMENT THERAPY • N=28
● HYPERTHYROID • N=27
▲ CONTROL • N= 30
PERCENTAGE OF TIME IN START DURING WHICH NO MOVEMENT OCCURRED
100
80
60
40
20
4
6
8
10
12
14
16
AGE OF PUPS (days)

Figure 24. Mean percentage of time spent in the start zone during which no movement occurred when pups were in the cool end of the thermal gradient is compared to the mean percentage when pups were in the warm end of the gradient, for each of the four treatment groups. Means and standard errors are presented in the Appendix, Table X.


PERCENTAGE OF TIME IN START DURING WHICH NO MOVEMENT OCCURRED
100
80
60
40
20
Hypothyroid
N = 18
Ⓐ
Ⓒ
Replacement Therapy
N= 28
cool start
warm start
Hyperthyroid
N = 27
Ⓑ
Ⓓ
Control
N = 30
4
8
12
16
AGE OF PUPS (days)

4) Pups in all treatment groups showed less movement at the warm end of the thermal gradient than at the cool end. The hypothyroid pups showed the greatest differences in responding at the two ends of the thermal gradient.

Discussion

The results from the present experiment support the general finding that the appearance of various behavioral responses is accelerated by hyperthyroidism and delayed by hypothyroidism. Furthermore, the findings demonstrate that pups can use olfactory cues in orientation and that hypothyroid pups may be able to use thermal cues in a kinetic orientation along thermal gradients.

The tests on the olfactory gradient suggest that pups in all of the treatment groups can respond to purely olfactory cues. In tests starting at the less intense end of the olfactory gradient, there were significant increases with age in the distance of the terminal location of the pup from the start zone. In contrast, when pups were started from the strong end of the olfactory gradient, they tended to stay in the start zone. On day 14, most of the control pups placed on the weak end of the olfactory gradient traveled directly to the strong end of the gradient and remained there (see Figure 18). Quite often, pups tried to climb over the side of the platform, apparently following the gradient directly to the source. Hyperthyroid pups showed this behavior on day 12 and hypothyroid pups on day 16. It is likely that the failure of replacement therapy pups to show orientation along the gradient was due to the termination of testing before the response had developed in these pups. From the home orientation data, it is clear that these pups do not differ significantly from controls, so that we may infer that had testing of these pups been possible on day 13 or 14, they might also have shown orientation along the gradient.

Hypothyroid pups showed a delay in the development of orientation in the home cage situation and along the olfactory gradient. Since orientation along the olfactory gradient occurred prior to eye opening, it seems that olfactory cues alone can serve in orientation. With the onset of visual functioning, there is most likely an integration of olfactory responsiveness with vision. It is quite possible that the deficit in responding in the home orientation task and on the olfactory gradient shown by hypothyroid animals is due in part to a delay in the development of motor ability. However, there are particularly compelling reasons to believe that the development of olfactory functioning might be delayed in these pups, thus accounting for the delay in the development of orientation using olfactory cues.

Much of the anatomical and physiological maturation of the olfactory bulb occurs postnatally in the rat. Altman and Das (1966) report that granule cell proliferation continues in the olfactory bulb until 20 to 22 days after birth. Furthermore, Salas, Guzman-Flores and Schapiro (1969) recorded spontaneous activity in the olfactory bulb and found that adult levels of activity were not reached until 24 days in the normal rat. Since thyroid hormone insufficiency or excess has the greatest effect on those portions of the brain which differentiate postnatally, e.g. cerebellum, it seems likely that the olfactory bulb may be affected by perinatal hypo- or hyperthyroidism. It is tempting to speculate that the migration of the granule cells from the ependymal and subependymal layers in the olfactory bulb is affected by hormone treatment, just as the migration of the granule cell population in the neonatal cerebellum is affected. If indeed there are deficits in the development of olfaction in these pups, it is possible that some of the observed behavioral deficits in hypothyroid pups might be due to a delayed development of the olfactory bulb. By comparing the behavior of hypothyroid animals with the behavior of normal pups with delayed development of olfaction it might be possible to determine whether some of the reported effects of congenital hypothyroidism might be mediated by the delayed olfactory functioning. Since the hypothyroid pups develop homing eventually, it would seem that they were quite sensitive to olfactory stimuli. However, it is possible that there are very subtle permanent effects on olfaction, involving either a decreased sensitivity to olfactory stimuli (i.e. a higher absolute threshold), or a decreased sensitivity to changes in stimulation (i.e. a larger difference threshold).

The results from the gradient tests support the findings that the pups were in fact not anosmic. Even on day 4 there were significant differences in the amount of "no movement" at the two ends of the olfactory gradient. In general, pups spent a higher percentage of time not moving at the strong end of the olfactory gradient. The differential response was most clearly shown by hypothyroid animals. The question arises as to how this finding can be reconciled with the apparent "activating" effect of being placed in the home quadrant that was noted when animals were tested for home orientation. One consideration was that the criteria were different in the two tests. In the home orientation test, the number of 15 sec intervals with 5 sec of "no locomotion" was counted, while in the gradient test the total number of 5 sec blocks in which there was no movement, locomotor or nonlocomotor, was counted. This means that the criterion was more rigorous for the gradient test. Yet, even with the stricter criterion, the rats showed significantly less movement at the strong end of the olfactory gradient than at the weak end. One might speculate that in the gradient test, the olfactory stimulus was dissociated from other stimuli normally present in the home cage situation, for example, thermal, tactile, auditory and possibly visual stimuli. The olfactory stimulus may have been novel to the pup in this type of situation, resulting in relative quiescence at the strong end of the gradient. Thus, the "activation" found in the home orientation test might have resulted from olfactory stimulation which was integrated with other types of stimulation normally found in the nest situation.

There was no evidence that the rat pups in the present experiment displayed the strong responsiveness to thermal gradients reported in hamster pups by Leonard (1974). There were significant increases in the terminal distance of the pup from the cool end of the start zone. However, the pups tended to move similar distances from the start when placed in the warm end of the gradient, suggesting that the pups were not discriminating between the cool and warm ends of the gradient. The fact that the rats moved significantly further when started at the weak end of the olfactory gradient than the cool end of the thermal gradient further suggests that the pups were not orienting along the thermal gradient, or at least not to the extent to which they oriented along the olfactory gradient.

The pups in the four treatment groups did show differential amounts of movement at the cool and warm ends of the gradient, confirming the findings of Fowler and Kellogg (1975). The hypothyroid pups showed the greatest difference and the hyperthyroid pups the least. The percentage of time spent not moving in the warm end of the gradient by hypothyroid pups was roughly equivalent to the percentage shown by controls 4 to 5 days earlier. This is especially interesting in light of Hamburgh's (1968) finding that hypothyroid rats were delayed approximately 5 days in the development of homeothermy in relation to controls. It is tempting to speculate that the differences in responsiveness shown by hypothyroid rats reflect their relative immaturity in terms of the development of homeothermy. Since Hamburgh suggests that normal thermoregulation may never be fully attained in hypothyroid animals, it might be interesting to determine whether older animals continue to show a differential responsiveness to thermal stimuli which differs from control animals.

Despite the fact that the pups showed no evidence of utilizing the thermal gradient to orient, the differential amounts of movement at the cool and warm ends of the gradient might be sufficient to permit orthokinetic orientation using thermal cues. The fact that hypothyroid pups show the largest difference in movement in warm and cool temperatures suggests that they may more readily show an orthokinetic orientation. It is likely that the 1 min time limit in the present experiment was not sufficient to permit the pups to move from the cool end to the warm end of the thermal gradient, given the large amount of random activity occurring in a kinesis. At any rate, there is a possibility that thermal cues can be used effectively if the pup is given adequate time to respond.

The question was raised in the introduction as to whether differences in the development of responsiveness to olfactory and thermal stimulation might account for the observed differences in the development of home orientation. It would appear that differences in the development of orientation using olfactory cues may account for some of the differences in homing. Hypothyroid pups showed a delay and hyperthyroid pups an acceleration in the emergence of the ability to orient along the olfactory gradient. This suggests that perinatal hormone deficiency or excess has effects on the development of the olfactory system. None of the treatment groups showed evidence of ability to utilize a thermal gradient, although all discriminated between the cool and warm ends of the gradient by showing differences in the amount of movement at the two ends of the gradient. The greatest differences in movement were shown by the hypothyroid pups, suggesting that they might be able to show a kinetic orientation along a thermal gradient.

V. CONCLUSIONS

During a limited portion of development in the rat pup, the brain becomes a critical target tissue for the action of thyroid hormones. The period of greatest vulnerability to hormone insufficiency or excess appears to coincide with the brain "growth spurt", the period of glial multiplication and neuronal differentiation occurring between birth and 2 to 3 weeks postnatally in the rat pup (Dobbing, 1973, 1974). Thyroid hormone manipulation during this critical period of brain development has ramifications at all levels of neuronal structure and function, and also affects the behavior of the young animal.

The change in neural organization induced by rendering an animal either hypo- or hyperthyroid did not appear to severely disrupt the behavioral organization studied in the present investigation. The synchrony between mother and offspring was maintained, so that the decline in some measures of maternal behavior was delayed in the mothers of the relatively immature hypothyroid pups. The behavior of the lactating female was appropriate to the developmental state of the hypothyroid pup, thus possibly alleviating some of the more severe effects of perinatal hypothyroidism. The integrated response of home orientation developed normally in hypothyroid animals, even though its emergence was delayed by several days. This suggests that the development of the sensory-motor integration underlying the response was delayed, but was not disrupted to the extent that homing could not occur. The emergence of the ability to utilize olfactory cues on a gradient was accelerated in hyperthyroid animals and delayed in hypothyroid animals, but once the response developed it did not appear to differ qualitatively from the response shown by control pups. Finally, pups in the four treatment groups showed differences in responsiveness on the thermal gradient which can be correlated with their development of homeothermy. Thus, hypothyroid pups, which show considerable delay in the development of thermoregulation, showed the greatest difference in activity as a function of temperature. This suggests that these pups might be particularly sensitive to thermal cues, perhaps utilizing behavioral thermoregulation to compensate for the delay in the development of intrinsic mechanisms of thermoregulation.

Thyroid hormones are believed to influence the timing of neural development, thus affecting the timing of the emergence of various behavioral responses. The behaviors themselves are apparently not disrupted; rather, the behaviors emerge normally at either earlier or later ages. Finally, the change in the appearance and behavior of the infant are most likely responsible for the subtle changes in the timing of the maternal behavior cycle. The changes in timing of both the mother-young interactions and the behavioral development of the pup may have profound influences on the later development of hypothyroid and hyperthyroid animals, perhaps by changing the normal temporal sequence of behavior development. For example, the failure of one sensory system to develop at the appropriate time could affect the extent to which that system is integrated with other sensory systems, the development of which was not delayed or was delayed to a different extent. Acceleration of development, as in hyperthyroidism, may similarly alter the relationship between different developing systems, possibly accounting for some of the reported differences in behavior as adults. Thus, it is important to consider not only the development of behavioral and sensory capacities by themselves, but also to consider the relationship between them.

The means by which thyroid hormones influence behavioral development are likely to be extremely complex, beginning at the cellular level. At present, we cannot easily trace the complex differences in behavior observed in hypothyroid and hyperthyroid animals to differences in neural organization. At best, we can analyze the behavior of such animals in an attempt to identify possible mediators of the numerous effects of thyroid hormones in development. One hopefully fruitful line of research, begun in the present study, would involve an assessment of the sensory capabilities of the hypo- and hyperthyroid animal; if deficits in sensory capacity are found, then we may be able to determine whether some of the other observed deficits in behavior might be due to the primary sensory deficit. Examination of the altered relationships between the various sensory and behavioral systems as they develop may also provide insight into possible causes of differences. Finally, an attempt can be made to identify the neural basis for the observed deficits, in an attempt to correlate neuronal structure and function.

APPENDIX

Table A

Mean Body Weight (in grams) of the Pups

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
1	5.7 ± 0.2	6.7 ± 0.3	5.7 ± 0.3	6.5 ± 0.1
2	6.2 ± 0.4	7.8 ± 0.3	6.4 ± 0.3	7.8 ± 0.1
4	8.0 ± 0.8	10.8 ± 0.3	8.4 ± 0.6	10.8 ± 0.2
6	10.2 ± 1.2	14.6 ± 0.6	10.9 ± 0.8	14.4 ± 0.2
8	12.7 ± 1.4	19.1 ± 0.8	14.0 ± 1.2	18.9 ± 0.6
10	15.2 ± 1.7	24.6 ± 1.0	17.9 ± 1.5	23.7 ± 0.8
12	17.7 ± 1.7	29.1 ± 1.2	21.7 ± 1.5	28.9 ± 1.0
14	20.6 ± 1.7	34.0 ± 1.4	25.4 ± 1.5	34.0 ± 1.2
16	22.6 ± 1.8	39.1 ± 1.5	29.0 ± 1.8	39.6 ± 1.3
18	24.9 ± 2.4	44.0 ± 2.2	32.9 ± 1.9	44.9 ± 1.4
20	26.5 ± 2.5	50.6 ± 2.5	37.9 ± 2.1	51.0 ± 2.0
22	28.8 ± 3.0	58.7 ± 2.8	43.3 ± 2.2	58.9 ± 2.2

Table B

Mean Nest Rating[a]

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 7)	Hyperthyroid (n = 8)	Replacement Therapy (n = 9)	Control (n = 8)
2	3.4 ± 0.3	3.6 ± 0.2	3.9 ± 0.1	3.1 ± 0.2
3	3.6 ± 0.3	3.5 ± 0.2	3.7 ± 0.2	3.6 ± 0.2
4	3.9 ± 0.2	3.5 ± 0.2	3.9 ± 0.1	3.8 ± 0.2
5	3.4 ± 0.3	3.0 ± 0.3	3.7 ± 0.2	3.5 ± 0.2
6	3.4 ± 0.2	3.0 ± 0.3	3.6 ± 0.3	3.4 ± 0.2
7	3.3 ± 0.2	3.0 ± 0.2	3.3 ± 0.2	3.3 ± 0.2
8	3.4 ± 0.3	2.9 ± 0.5	3.6 ± 0.2	3.3 ± 0.3
9	3.7 ± 0.2	2.6 ± 0.3	3.7 ± 0.2	2.9 ± 0.2
10	3.7 ± 0.2	2.6 ± 0.3	3.1 ± 0.3	2.9 ± 0.2
11	3.3 ± 0.4	2.5 ± 0.4	2.9 ± 0.3	2.8 ± 0.3
12	3.7 ± 0.2	2.3 ± 0.4	3.1 ± 0.3	2.5 ± 0.2
13	3.4 ± 0.2	2.1 ± 0.1	2.8 ± 0.4	2.4 ± 0.3
14	3.6 ± 0.2	1.8 ± 0.2	2.4 ± 0.3	1.8 ± 0.2
15	3.3 ± 0.2	1.4 ± 0.3	2.0 ± 0.3	1.6 ± 0.2
16	2.9 ± 0.3	1.3 ± 0.2	2.2 ± 0.2	1.9 ± 0.2
17	3.0 ± 0.2	1.1 ± 0.2	1.9 ± 0.3	1.8 ± 0.3
18	2.6 ± 0.2	1.0 ± 0.2	1.6 ± 0.3	1.5 ± 0.2
19	2.4 ± 0.2	0.8 ± 0.3	1.2 ± 0.2	1.0 ± 0.3
20	2.1 ± 0.5	0.5 ± 0.3	1.0 ± 0.3	1.4 ± 0.2
21	2.3 ± 0.2	0.5 ± 0.2	0.8 ± 0.2	1.3 ± 0.2
22	2.3 ± 0.4	0.5 ± 0.3	0.6 ± 0.3	1.4 ± 0.2

[a] Nest rating scale devised by Seitz (1958):

0 - no nest
1 - shavings trampled down in one corner of cage
2 - shavings pushed aside to make bare spot in corner
3 - low ring of shavings around bare spot in corner
4 - all shavings piled into high ring around bare spot

Table C

Mean Percentage of Intervals
During which Nursing Occurred
(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 7)	Hyperthyroid (n = 8)	Replacement Therapy (n = 9)	Control (n = 8)
2	91.1 ± 7.9	93.8 ± 4.2	94.0 ± 5.8	96.8 ± 2.2
4	95.4 ± 2.3	72.8 ±10.6	89.8 ± 6.7	93.4 ± 4.6
6	80.9 ± 7.7	90.1 ± 2.8	61.9 ±16.5	81.1 ±10.7
8	73.9 ±12.0	68.2 ±11.0	72.2 ±10.4	90.1 ± 3.9
10	93.5 ± 5.2	73.6 ±12.1	82.9 ±10.1	77.1 ± 7.2
12	82.7 ±10.4	49.4 ±12.6	57.9 ±12.6	78.7 ± 8.4
14	79.3 ± 9.4	33.6 ±10.3	43.1 ± 5.9	68.9 ±13.1
16	66.9 ±14.8	42.0 ±13.2	40.5 ±13.5	29.0 ±13.8
18	64.5 ±12.4	9.1 ± 5.1	47.9 ±14.8	37.7 ±11.7
20	44.7 ±12.0	21.2 ± 8.1	21.1 ± 5.7	42.1 ±11.6
22	56.6 ±15.3	34.0 ±10.8	8.5 ± 5.8	48.7 ± 8.3

Table D

Mean Number of Intervals in Which Grooming Occurred [a]

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 7)	Hyperthyroid (n = 8)	Replacement Therapy (n = 9)	Control (n = 8)
2	5.1 ± 1.9	5.0 ± 1.5	1.9 ± 1.4	5.9 ± 2.6
4	5.0 ± 2.9	7.3 ± 2.4	2.9 ± 1.3	8.1 ± 3.6
6	5.4 ± 1.8	11.9 ± 2.3	2.2 ± 1.0	6.9 ± 2.1
8	4.5 ± 1.4	10.3 ± 2.8	5.4 ± 1.6	6.0 ± 2.5
10	5.5 ± 2.3	11.4 ± 1.9	5.1 ± 1.6	12.4 ± 3.5
12	6.7 ± 1.7	4.9 ± 1.3	4.6 ± 1.8	10.3 ± 3.2
14	3.7 ± 1.9	3.5 ± 1.1	6.6 ± 1.5	0.9 ± 0.6
16	1.9 ± 0.4	2.1 ± 1.3	3.1 ± 1.2	1.3 ± 1.3
18	5.4 ± 2.0	1.8 ± 1.0	3.0 ± 1.8	1.3 ± 0.6
20	1.9 ± 1.1	2.3 ± 1.1	5.1 ± 2.1	1.8 ± 1.2
22	3.0 ± 1.0	2.6 ± 0.9	3.8 ± 1.8	1.9 ± 1.3

[a] Maximum number of intervals = 60.

Table E

Mean Number of Intervals in Which Contact Occurred[a]

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 7)	Hyperthyroid (n = 8)	Replacement Therapy (n = 9)	Control (n = 8)
2	59.8 ± 0.2	57.2 ± 2.0	56.7 ± 3.5	60.0 ± 0.0
4	59.9 ± 0.1	47.6 ± 6.3	56.7 ± 3.5	58.3 ± 1.8
6	54.5 ± 3.3	58.4 ± 0.9	47.2 ± 7.1	54.4 ± 3.9
8	48.0 ± 6.8	45.6 ± 6.6	47.3 ± 5.7	58.3 ± 1.3
10	57.5 ± 2.7	50.0 ± 7.2	51.9 ± 5.2	54.1 ± 4.2
12	50.3 ± 7.3	34.1 ± 8.5	38.6 ± 7.5	53.4 ± 3.9
14	50.4 ± 5.8	37.4 ± 5.9	32.3 ± 4.6	51.8 ± 5.1
16	43.6 ± 8.4	38.6 ± 5.4	36.6 ± 6.1	26.9 ± 8.3
18	43.8 ± 6.4	28.1 ± 6.2	43.1 ± 7.5	52.9 ± 3.7
20	42.8 ± 5.8	46.0 ± 6.3	38.6 ± 5.4	45.6 ± 6.2
22	41.6 ± 7.9	52.5 ± 3.8	27.9 ± 6.1	49.6 ± 4.5

[a] Maximum number of intervals = 60.

Table F

Mean Percentage of Pups

Not in Contact with Female

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 7)	Hyperthyroid (n = 8)	Replacement Therapy (n = 9)	Control (n = 8)
2	9.4 ± 6.8	6.0 ± 3.4	8.8 ± 5.9	1.3 ± 1.2
4	6.4 ± 2.8	23.5 ±11.0	13.0 ± 6.1	4.8 ± 3.8
6	12.1 ± 5.5	6.7 ± 2.4	26.1 ±12.5	10.0 ± 4.5
8	25.0 ±13.4	28.7 ±10.7	31.1 ±12.6	8.8 ± 3.3
10	8.1 ± 6.1	21.0 ±11.8	15.7 ± 9.0	19.3 ± 6.3
12	17.2 ± 8.8	50.9 ±12.4	40.0 ±11.8	20.2 ± 7.1
14	18.3 ± 9.1	57.4 ±10.7	50.1 ± 8.4	27.0 ±10.0
16	30.2 ±14.4	58.6 ± 9.1	51.3 ±11.6	66.2 ±12.1
18	30.3 ±10.9	77.8 ± 9.9	44.2 ±13.2	43.8 ± 8.6
20	45.9 ± 8.7	57.2 ± 9.7	57.1 ± 9.0	43.2 ±12.1
22	44.6 ±11.9	47.8 ± 6.2	73.7 ± 9.3	42.0 ± 8.4

Table G

Mean Latency (in sec) to Contact Pups in the Retrieval Test[a]

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 7)	Hyperthyroid (n = 8)	Replacement Therapy (n = 9)	Control (n = 8)
2	110.4 ± 53.2	97.0 ± 37.3	207.4 ± 52.5	158.5 ± 49.4
4	79.9 ± 44.3	75.4 ± 32.1	193.7 ± 49.0	52.9 ± 26.3
6	33.7 ± 8.9	42.4 ± 26.8	137.3 ± 49.3	54.8 ± 34.7
8	92.6 ± 48.6	84.0 ± 48.7	77.7 ± 33.3	18.3 ± 6.0
10	41.6 ± 26.2	91.3 ± 49.4	46.9 ± 36.2	47.8 ± 38.6
12	64.0 ± 43.5	36.6 ± 14.8	41.0 ± 36.8	85.5 ± 50.3

[a] A maximum latency of 300 sec was assigned to mothers which did not contact pups during the 5 min test.

Table H

Mean Latency (in sec) to Retrieve the First Pup on the Retrieval Test

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 7)	Hyperthyroid (n = 8)	Replacement Therapy (n = 9)	Control (n = 8)
2	300.0 ± 0.0	229.1 ± 40.7	283.3 ± 17.7	216.5 ± 44.5
4	264.4 ± 38.4	221.1 ± 43.5	249.2 ± 36.8	159.8 ± 47.6
6	285.3 ± 15.9	162.5 ± 55.8	215.1 ± 45.8	127.5 ± 53.2
8	261.3 ± 39.7	161.9 ± 55.9	199.0 ± 43.2	120.6 ± 56.2
10	243.3 ± 44.4	159.3 ± 47.5	215.1 ± 40.2	180.0 ± 50.8
12	237.6 ± 43.9	176.9 ± 50.5	254.6 ± 31.9	224.5 ± 47.0

[a] A maximum latency of 300 sec was assigned to mothers which did not retrieve a pup during the 5 min test.

Table I

Mean Percentage of Pups Terminating in the Home When Started in the Adjacent Corner

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.6 ± 3.2
6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 2.7
8	3.6 ± 3.9	36.9 ± 13.8	13.0 ± 11.7	22.1 ± 9.5
10	0.0 ± 0.0	67.7 ± 14.7	43.3 ± 11.5	55.4 ± 13.0
12	9.5 ± 10.3	87.5 ± 5.6	79.8 ± 11.7	84.0 ± 5.5
14	26.4 ± 11.7	80.8 ± 8.5	93.7 ± 3.4	88.5 ± 10.0
16	51.0 ± 14.5	72.1 ± 6.0	71.7 ± 8.2	80.4 ± 7.8
18	62.9 ± 9.9	74.6 ± 6.7	58.1 ± 14.4	64.2 ± 10.4
20	87.6 ± 6.6	41.0 ± 9.0	52.0 ± 9.8	67.5 ± 12.1
22	52.4 ± 14.0	44.6 ± 6.5	69.4 ± 4.1	46.0 ± 13.5

Table J

Mean Percentage of Pups Terminating in the Home When Started in the Diagonal Corner

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8	0.0 ± 0.0	6.3 ± 6.7	1.9 ± 2.0	4.6 ± 3.2
10	0.0 ± 0.0	50.6 ± 13.1	19.3 ± 6.2	28.3 ± 6.8
12	0.0 ± 0.0	82.3 ± 9.0	65.9 ± 9.5	68.5 ± 9.5
14	26.4 ± 11.7	81.9 ± 5.9	77.6 ± 7.3	72.3 ± 10.8
16	20.9 ± 12.4	56.0 ± 10.8	55.7 ± 11.0	80.8 ± 9.0
18	64.0 ± 16.3	63.8 ± 4.1	62.0 ± 6.0	65.8 ± 8.0
20	67.1 ± 8.3	50.8 ± 6.5	67.2 ± 10.5	69.2 ± 5.9
22	51.9 ± 7.9	58.1 ± 8.1	69.8 ± 6.3	40.8 ± 6.4

Table K

Mean Percentage of Pups Terminating in the Home When Started in the Home Corner

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	83.3 ± 8.8	93.7 ± 4.7	93.1 ± 3.7	77.3 ± 5.9
6	90.5 ± 10.3	97.9 ± 2.2	100.0 ± 0.0	95.4 ± 3.2
8	90.5 ± 6.6	89.6 ± 6.7	97.2 ± 2.9	91.7 ± 6.7
10	95.2 ± 5.1	94.8 ± 3.7	92.6 ± 7.9	95.4 ± 3.2
12	100.0 ± 0.0	92.9 ± 5.5	93.7 ± 4.9	100.0 ± 0.0
14	90.5 ± 6.6	83.5 ± 6.8	84.6 ± 5.6	97.9 ± 2.2
16	85.7 ± 10.7	61.0 ± 8.6	67.6 ± 8.6	73.8 ± 6.9
18	90.7 ± 4.8	63.7 ± 8.6	61.3 ± 7.0	60.0 ± 8.9
20	65.2 ± 14.1	59.6 ± 10.6	52.8 ± 6.6	70.2 ± 6.3
22	71.0 ± 10.1	54.6 ± 9.7	51.8 ±10.5	57.9 ± 9.2

Table L

Mean Number of 15 Sec Intervals in which
5 Sec of "No Locomotion" Occurred when
Pups Started in the Adjacent Quadrant
(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	7.7 ± 0.1	7.5 ± 0.2	7.5 ± 0.2	7.1 ± 0.3
6	7.4 ± 0.6	7.7 ± 0.2	7.8 ± 0.1	7.7 ± 0.1
8	7.3 ± 0.4	6.4 ± 0.5	6.8 ± 0.6	6.3 ± 0.4
10	7.0 ± 0.4	4.5 ± 0.4	5.3 ± 0.8	4.9 ± 0.4
12	6.6 ± 0.7	2.2 ± 0.6	3.0 ± 0.6	2.4 ± 0.3
14	4.6 ± 1.0	1.0 ± 0.3	1.1 ± 0.3	0.8 ± 0.2
16	3.2 ± 0.7	0.8 ± 0.2	0.6 ± 0.1	1.0 ± 0.2
18	1.9 ± 0.7	1.1 ± 0.3	0.9 ± 0.2	1.0 ± 0.2
20	1.7 ± 0.5	2.2 ± 0.4	1.1 ± 0.3	1.5 ± 0.4
22	1.4 ± 0.6	1.7 ± 0.2	1.8 ± 0.5	2.0 ± 0.5

Table M

Mean Number of 15 Sec Intervals in which 5 Sec of "No Locomotion" Occurred when Pups Started in the Diagonal Quadrant

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	7.2 ± 0.4	7.7 ± 0.2	7.3 ± 0.3	7.5 ± 0.2
6	7.3 ± 0.4	7.6 ± 0.2	7.8 ± 0.1	7.9 ± 0.0
8	7.3 ± 0.4	6.3 ± 0.7	7.1 ± 0.3	6.6 ± 0.3
10	7.0 ± 0.6	3.9 ± 0.6	4.9 ± 0.7	4.4 ± 0.9
12	6.0 ± 0.9	1.9 ± 0.4	2.7 ± 0.4	1.9 ± 0.3
14	4.4 ± 0.8	1.1 ± 0.2	1.2 ± 0.3	1.0 ± 0.3
16	2.6 ± 0.5	0.7 ± 0.2	0.5 ± 0.3	0.7 ± 0.2
18	2.3 ± 0.5	0.9 ± 0.2	0.7 ± 0.2	1.0 ± 0.2
20	1.7 ± 0.5	2.4 ± 0.4	1.0 ± 0.3	1.3 ± 0.3
22	1.3 ± 0.4	2.6 ± 0.3	1.6 ± 0.4	1.8 ± 0.3

Table N

Mean Number of 15 Sec Intervals in which 5 Sec of "No Locomotion" Occurred when Pups Started in the Home Quadrant

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	5.4 ± 0.6	6.5 ± 0.4	6.1 ± 0.5	5.6 ± 0.5
6	5.3 ± 0.8	6.9 ± 0.2	7.4 ± 0,2	5.9 ± 0.7
8	6.3 ± 0.8	6.6 ± 0.3	6.7 ± 0.5	5.9 ± 0.6
10	5.5 ± 0.8	5.2 ± 0.4	5.6 ± 0.5	4.8 ± 0.5
12	5.2 ± 0.8	2.2 ± 0.7	2.7 ± 0.4	2.8 ± 0.4
14	2.8 ± 0.5	0.6 ± 0.2	0.9 ± 0.2	0.9 ± 0.3
16	2.4 ± 0.6	0.8 ± 0.2	0.6 ± 0.2	0.8 ± 0.2
18	1.8 ± 0.6	1.1 ± 0.3	0.8 ± 0.2	1.1 ± 0.4
20	1.2 ± 0.4	2.3 ± 0.5	1.4 ± 0.2	1.6 ± 0.3
22	1.3 ± 0.4	2.3 ± 0.4	1.9 ± 0.5	2.0 ± 0.5

Table O

Mean Number of Quadrants Crossed When Pups were Started in the Adjacent Corner

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2
6	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.6	0.0 ± 0.0
8	0.1 ± 0.1	0.5 ± 0.2	0.3 ± 0.2	0.5 ± 0.2
10	0.0 ± 0.0	1.1 ± 0.3	0.7 ± 0.2	1.6 ± 0.6
12	1.0 ± 0.9	4.5 ± 1.0	3.2 ± 0.9	3.2 ± 0.7
14	1.2 ± 0.4	9.5 ± 1.5	9.8 ± 1.5	8.7 ± 1.1
16	2.3 ± 0.7	21.3 ± 2.1	19.3 ± 3.1	13.5 ± 2.0
18	5.2 ± 1.6	19.0 ± 1.4	22.0 ± 1.6	19.0 ± 2.3
20	9.0 ± 2.2	14.9 ± 1.1	19.1 ± 2.0	17.7 ± 1.6
22	11.9 ± 2.4	17.1 ± 1.2	14.8 ± 1.5	12.2 ± 1.1

Table P

Mean Number of Quadrants Crossed When Pups were Started in the Diagonal Corner

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.2
6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8	0.1 ± 0.1	0.5 ± 0.2	0.2 ± 0.2	0.2 ± 0.1
10	0.0 ± 0.0	2.0 ± 0.4	1.2 ± 0.5	1.9 ± 0.5
12	0.5 ± 0.5	5.4 ± 1.1	3.8 ± 0.7	3.8 ± 0.9
14	1.3 ± 0.5	8.7 ± 1.1	8.7 ± 1.1	7.8 ± 1.1
16	2.3 ± 0.8	19.1 ± 1.5	20.0 ± 2.6	14.5 ± 2.3
18	4.5 ± 1.4	21.2 ± 1.5	20.0 ± 1.7	19.6 ± 1.5
20	9.0 ± 2.6	14.4 ± 2.2	19.5 ± 1.3	15.7 ± 1.7
22	10.0 ± 2.5	11.6 ± 1.2	17.2 ± 1.8	13.0 ± 1.3

Table Q

Mean Number of Quadrants Crossed When Pups were Started in the Home Corner

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	0.2 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	0.4 ± 0.2
6	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
8	0.4 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.5 ± 0.2
10	0.2 ± 0.1	1.2 ± 0.4	0.6 ± 0.2	0.7 ± 0.4
12	0.4 ± 0.3	3.7 ± 1.1	1.8 ± 0.4	2.8 ± 0.8
14	1.5 ± 0.7	8.1 ± 1.5	8.8 ± 1.5	7.0 ± 1.3
16	2.4 ± 0.7	17.5 ± 2.1	14.8 ± 2.1	11.5 ± 1.6
18	3.9 ± 1.1	17.9 ± 1.9	22.7 ± 1.8	17.4 ± 1.9
20	8.3 ± 2.3	12.8 ± 1.6	17.8 ± 0.7	13.5 ± 1.7
22	12.3 ± 2.7	12.3 ± 1.2	12.5 ± 1.6	11.6 ± 1.2

Table R

Mean Latency (in sec) to Leave the Adjacent Quadrant[a]

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	120.0 ± 0.0	120.0 ± 0.0	118.6 ± 1.5	109.9 ± 4.9
6	120.0 ± 0.0	120.0 ± 0.0	119.3 ± 0.7	117.4 ± 2.8
8	115.2 ± 5.1	88.0 ±10.9	106.0 ±10.1	99.3 ± 9.4
10	120.0 ± 0.0	50.8 ±10.5	84.5 ±11.3	65.7 ±12.4
12	113.1 ± 7.4	14.4 ± 7.1	33.5 ±11.5	22.8 ± 9.2
14	81.9 ±12.7	4.1 ± 0.6	11.7 ± 3.2	6.5 ± 0.5
16	50.0 ±11.5	2.3 ± 0.5	3.6 ± 0.5	5.1 ± 0.9
18	38.3 ±14.9	2.0 ± 0.5	2.1 ± 0.2	2.6 ± 0.2
20	15.4 ± 5.6	1.8 ± 0.2	2.2 ± 0.5	3.5 ± 0.7
22	15.9 ± 7.0	1.9 ± 0.2	1.8 ± 0.2	4.5 ± 2.1

[a] A maximum latency of 120 sec was assigned to pups which did not leave the test quadrant during the 2 min test.

Table S

Mean Latency (in sec) to Leave the Diagonal Quadrant[a]

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	120.0 ± 0.0	120.0 ± 0.0	118.6 ± 1.0	113.7 ± 4.6
6	120.0 ± 0.0	120.0 ± 0.0	120.0 ± 0.0	120.0 ± 0.0
8	113.9 ± 4.4	93.9 ± 12.7	114.3 ± 4.3	102.1 ± 11.4
10	120.0 ± 0.0	61.5 ± 13.1	81.9 ± 12.6	81.0 ± 12.2
12	114.0 ± 5.7	15.3 ± 3.2	38.1 ± 10.9	35.6 ± 11.4
14	73.9 ± 14.1	10.4 ± 2.8	22.3 ± 7.3	11.3 ± 2.8
16	72.5 ± 11.6	1.9 ± 0.3	4.7 ± 0.8	3.2 ± 0.5
18	49.3 ± 17.6	2.1 ± 0.2	2.0 ± 0.3	2.1 ± 0.2
20	17.0 ± 5.9	1.5 ± 0.2	1.7 ± 0.3	3.5 ± 0.9
22	24.1 ± 7.3	1.6 ± 0.2	1.8 ± 0.3	4.9 ± 2.1

[a] A maximum latency of 120 sec was assigned to pups which did not leave the test quadrant during the 2 min test.

Table T

Mean Latency (in sec) to Leave the Home Quadrant[a]

Age (days)	Hypothyroid (n = 27)	Hyperthyroid (n = 42)	Replacement Therapy (n = 43)	Control (n = 44)
4	108.7 ± 6.1	116.8 ± 2.3	118.0 ± 1.1	97.3 ± 5.9
6	106.1 ± 8.2	117.8 ± 2.4	120.0 ± 0.0	112.0 ± 5.8
8	112.4 ± 4.8	103.9 ± 9.4	115.4 ± 2.7	96.2 ± 9.3
10	112.2 ± 5.7	83.3 ± 10.1	100.4 ± 7.4	98.7 ± 7.8
12	112.6 ± 5.3	47.2 ± 9.1	82.3 ± 6.8	58.5 ± 9.9
14	82.5 ± 11.9	31.5 ± 7.7	34.5 ± 6.5	35.3 ± 10.9
16	65.7 ± 11.4	13.8 ± 8.1	19.8 ± 6.3	17.8 ± 4.7
18	46.9 ± 13.6	10.2 ± 4.9	3.7 ± 0.9	9.6 ± 4.3
20	27.3 ± 12.8	18.3 ± 4.9	6.2 ± 2.6	18.0 ± 9.0
22	29.2 ± 11.7	18.5 ± 7.4	16.5 ± 5.1	13.4 ± 3.8

[a] A maximum latency of 120 sec was assigned to pups which did not leave the test quadrant during the 2 min test.

Table U

Distance from the End of the Olfactory Gradient (in cm)

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 18)	Hyperthyroid (n = 27)	Replacement Therapy (n = 28)	Control (n = 30)
Started at the Weak End				
4	0.0 ± 0.0	0.5 ± 0.3	0.1 ± 0.0	0.1 ± 0.0
6	0.2 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
8	1.0 ± 0.2	0.9 ± 0.2	0.5 ± 0.3	0.8 ± 0.3
10	0.9 ± 0.2	4.4 ± 1.4	1.2 ± 0.4	3.7 ± 1.3
12	1.4 ± 0.7	17.1 ± 2.0	6.0 ± 0.4	9.1 ± 2.1
14	3.6 ± 1.1	---- ---	--- ---	19.7 ± 1.6
16	13.0 ± 3.7	---- ---	--- ---	---- ---
Started at the Strong End				
4	0.1 ± 0.1	0.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
6	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
8	0.9 ± 0.5	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0.2
10	0.0 ± 0.0	0.4 ± 0.1	0.6 ± 0.3	1.2 ± 0.9
12	2.1 ± 1.1	0.2 ± 0.2	3.0 ± 0.8	1.4 ± 0.8
14	2.2 ± 1.4	--- ---	--- ---	1.0 ± 0.9
16	0.2 ± 0.8	--- ---	--- ---	--- ---

Table V

Percentage of the Time in the Start During Which No Movement Occurred - Olfactory Gradient

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 18)	Hyperthyroid (n = 27)	Replacement Therapy (n = 28)	Control (n = 30)
Started at the Weak End				
4	70.1 ± 5.9	59.8 ± 6.6	62.6 ±10.4	75.6 ± 3.7
6	31.7 ±10.8	13.1 ± 6.8	17.2 ± 5.7	30.5 ± 0.6
8	12.0 ± 7.4	9.7 ± 4.7	8.5 ± 2.2	8.9 ± 6.7
10	13.4 ± 6.0	3.4 ± 1.0	12.0 ± 7.4	7.1 ± 5.5
12	5.5 ± 3.5	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.7
14	3.0 ± 2.7	--- ---	--- ---	0.0 ± 0.0
16	1.1 ± 2.0	--- ---	--- ---	--- ---
Started at the Strong End				
4	81.3 ± 6.8	67.8 ± 4.6	74.3 ± 7.2	84.1 ± 3.0
6	48.8 ±13.0	19.2 ± 7.8	21.9 ± 7.8	46.2 ± 6.9
8	22.6 ± 8.2	19.1 ± 3.3	11.6 ± 2.3	10.3 ± 3.8
10	42.3 ± 6.6	15.3 ± 6.0	8.2 ± 4.1	21.3 ± 6.7
12	10.7 ± 6.4	0.8 ± 0.7	0.3 ± 0.3	2.8 ± 1.4
14	7.0 ± 3.7	--- ---	--- ---	0.0 ± 0.0
16	1.1 ± 2.0	--- ---	--- ---	--- ---

Table W

Distance from the End of the Thermal Gradient (in cm)

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 18)	Hyperthyroid (n = 27)	Replacement Therapy (n = 28)	Control (n = 30)
Started at the Cool End				
4	0.1 ± 0.0	0.3 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
6	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
8	0.2 ± 0.1	2.2 ± 0.5	0.5 ± 0.2	0.8 ± 0.4
10	1.4 ± 0.4	3.9 ± 1.2	1.2 ± 0.4	2.6 ± 0.5
12	1.7 ± 0.8	9.6 ± 0.7	3.2 ± 0.6	5.8 ± 0.9
14	2.9 ± 0.9	--- ---	--- ---	11.8 ± 2.3
16	11.3 ± 4.5	--- ---	--- ---	---- ---
Started at the Warm End				
4	0.2 ± 0.2	0.5 ± 0.3	0.0 ± 0.0	0.1 ± 0.0
6	0.0 ± 0.0	0.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
8	0.1 ± 0.1	1.9 ± 1.0	0.4 ± 0.2	1.6 ± 0.6
10	2.1 ± 1.8	3.6 ± 0.8	1.7 ± 0.6	3.5 ± 1.5
12	0.3 ± 0.3	5.7 ± 1.2	5.4 ± 1.6	4.4 ± 1.6
14	0.0 ± 0.0	--- ---	--- ---	8.0 ± 2.2
16	5.6 ± 2.2	--- ---	--- ---	--- ---

Table X

Percentage of the Time in the Start During Which No Movement Occurred - Thermal Gradient

(Mean ± Standard Error)

Age (days)	Hypothyroid (n = 18)	Hyperthyroid (n = 27)	Replacement Therapy (n = 28)	Control (n = 30)
Started at the Cool End				
4	82.7 ± 6.8	48.2 ± 4.1	63.6 ± 11.0	73.0 ± 7.3
6	41.9 ± 11.8	19.4 ± 7.5	23.7 ± 7.5	31.2 ± 6.8
8	17.8 ± 10.8	7.9 ± 3.3	5.4 ± 3.1	4.9 ± 1.8
10	19.5 ± 7.7	9.8 ± 7.1	13.3 ± 5.3	7.5 ± 2.7
12	12.7 ± 6.4	2.8 ± 2.4	0.0 ± 0.0	0.0 ± 0.0
14	8.7 ± 4.9	--- ---	--- ---	0.0 ± 0.0
16	1.9 ± 1.8	--- ---	--- ---	--- ---
Started at the Warm End				
4	74.0 ± 14.1	35.4 ± 8.6	80.9 ± 7.0	48.3 ± 5.1
6	67.9 ± 8.4	28.3 ± 8.5	47.4 ± 4.7	65.4 ± 2.8
8	69.6 ± 13.4	31.8 ± 7.2	20.2 ± 2.3	29.6 ± 6.9
10	63.8 ± 8.6	17.3 ± 5.9	21.0 ± 4.4	23.2 ± 5.6
12	46.8 ± 5.6	6.4 ± 4.4	0.0 ± 0.0	10.9 ± 3.0
14	31.0 ± 8.9	--- ---	--- ---	2.8 ± 2.3
16	9.1 ± 5.1	--- ---	--- ---	--- ---

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