Role of maternal biochemistry in fetal brain development: effect of maternal thyroidectomy on behaviour and biogenic amine metabolism in rat progeny

Arnold J. Friedhoff, Jeannette C. Miller, Mary Armour, Jack W. Schweitzer and Sandhya Mohan

Barbara Jonas Center for the Study and Treatment of Children at Risk, Department of Psychiatry, New York University School of Medicine, 550 First Avenue, New York, NY 10016

Abstract

Few studies have addressed the role of biochemicals of maternal origin on fetal neurodevelopment and behavioural outcome. Thyroid deficiency in the thyroidectomized pregnant rat provides an excellent model to study fetal effects of maternal chemistry, as this condition is known to be associated with deficits in motor and cognitive behaviour in human offspring. Based on evidence that thyroid hormone of maternal origin may be an important determinant in regulating these behaviours, we assessed neurobehaviours and regional brain biogenic amine levels in offspring of rats thyroidectomized (Tx) prior to conception. Cross-fostering techniques were used to isolate fetal effects of maternal thyroid deficiency from possible neonatal effects during nursing by thyroid-deficient dams. The progeny of Tx dams showed significant deficits in maze learning, were less cautious in emotionality testing, and were more active in open-field exploration. Tx females appeared to be more vulnerable to the effects on learning. Learning in Tx males was only slightly impaired. Serotonin and dopamine metabolism was also affected in a brain region-specific manner in Tx progeny. Levels of 5-HIAA were reduced in the olfactory tubercle and cortex. HVA levels were lower in olfactory tubercle, but were elevated in the hippocampus. As these neurotransmitters play a functional role in activity, mood and learning, the findings may be pertinent to the observed behavioural impairments. The results are consistent with the hypothesis that an adequate in utero thyroid hormone environment may be essential for early fetal neurodevelopment even if the fetus is euthyroid.

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Introduction

We have proposed that maternally transmitted biochemicals have an important role in fetal neurodevelopment (Miller et al., 1991). The impact of maternal biochemistry on fetal brain development can be illustrated by changes in the function of maternal systems such as the thyroid. Epidemiological studies have suggested an association between maternal hypothyroxinaemia and cognitive/motor dysfunction in human offspring (Man et al., 1991). Previously, studies of the effects of thyroid hormones (THs) on neurodevelopment in rats focused on methods that produced neonatal hypothyroidism. Under these conditions severe deficiencies in structural, biochemical and other neural aspects of development have been reported (Dussault and Ruel, 1987; Morreale de Escobar et al., 1983). The focus on postnatal hypothyroidism was due to the original belief that the critical period for TH effects on brain development occurred after the establishment of fetal thyroid hormonogenesis (Oklund and Timiras, 1977), now known to occur in late gestation (Morreale de Escobar et al., 1983, 1985).

More recently, studies have been conducted to determine whether THs (particularly T4) have a function in early fetal neurodevelopment. A number of different experimental approaches have been used. In general, fetuses and offspring of hypothyroxinaemic rat dams exhibit deficiencies in cerebral growth, myelogenesis, and enzymes and glycoproteins necessary for synapto-
genesis. These impairments have been implicated in the behavioural dysfunction and impaired cognitive performance seen in progeny of hypothyroxinaemic dams (Porterfield and Hendrich, 1993; Sinha et al., 1992, for reviews). Disturbances in neurotransmitter function have also been implicated in the behavioural changes seen in progeny of hypothyroxinaemic dams (Attree et al., 1992; Evans et al., 1999; Sinha et al., 1991).

It is now known that T4 and T3 can be detected in fetal brain in midgestation, prior to the onset of fetal thyroid autonomy (Obregon et al., 1984; Porterfield and Hendrich, 1992; Ruiz de Ona et al., 1991). The observation that levels of brain T4 and T3 are significantly lower in midgestation fetuses of thyroidectomized (Tx) rat dams (Ruiz de Ona et al., 1991) supports the proposal that midgestational thyroid hormone levels in fetal brain are of maternal origin (Porterfield and Hendrich, 1992.

In an earlier study, Hendrich et al. (1984) showed that the progeny of radiothyroidectomized rats were hyperactive and showed severe deficits in learning; however, the radiothyroidectomized dams received T4 postpartum, throughout a 22-d lactation period in order to ensure adequate nursing of the progeny. It is not clear from the data whether exposure of the progeny during nursing to T4 contributed to the behavioural disturbances observed or were able to correct the adverse effects produced during fetal development. In order to focus on the early fetal effects of maternal hypothyroxinaemia we utilized a maternal thyroidectomy rat model without T4 replacement prior to mating, and cross-fostering of the progeny with untreated nursing dams. The progeny were assessed for behaviours reflecting emotional responsiveness, activity level and learning, and for brain region-specific biogenic amine metabolism.

**Methods**

**Animals**

Female rats of the Sprague–Dawley strain (Taconic Farms, Germantown, NY) were surgically thyroidectomized at the breeders. Thyroidectomy consisted of removal of the thyroid gland and replacement of the parathyroid glands. The animals were allowed to recover 2 wk post-surgery and the serum T3 and T4 levels determined by the breeder to ensure a hypothyroxinaemic state. No additional treatment or T4 replacement was provided prior to placing the hypothyroxinaemic females with untreated male rats for mating. Although the breeder indicated that the impregnation rate for hypothyroid females would be very low, they were able to provide impregnated Tx dams \( (n = 9) \). Sham-operated rats \( (n = 6) \) were provided as controls. The animals were shipped to our animal

facilities on gestational day 8. The pregnant rats were housed individually at constant room temperature \( (21 \pm 1 \, ^\circ\text{C}) \), with a 12:12 h light–dark cycle. Rats had free access to water and rat chow that contained adequate iodine. Following birth, the progeny from the control (6 litters with 9 \( \pm \) 1 pups) and Tx dams (5 litters with 8 \( \pm \) 1 pups; 3 Tx dams did not come to term and 1 delivered only 3 pups which did not survive after cross-fostering past postnatal (PN) day 2). The number of pups per litter were standardized to \( n = 8 \). Both control and Tx progeny were cross-fostered at birth to a separate group of untreated nursing dams also obtained from Taconic Farms. Cross-fostering was carried out to ensure adequate lactation and eliminate other possible effects of the hypothyroxinaemic state of the Tx dams on the progeny during nursing. Gender status was determined on PN day 1 by measuring the ano-genital distance. At weaning, PN day 21, the animals were randomized within treatment groups, housed by gender 3–4 per cage, and maintained under standard laboratory conditions throughout the study. Control progeny from 6 litters consisted of 21 males and 27 females. The Tx progeny from 5 litters consisted of 20 males and 20 females. Some of these progeny were used for the behavioural assessments and others sacrificed for neurochemical analyses and T3 and T4 analyses at the ages indicated below. The remaining control and Tx progenies were utilized for second-generation breeding for a future study.

These animal studies were approved by the New York University Medical Center Institutional Care and Use Committee and were conducted according to the National Institutes of Health ‘Guide for the Care and Use of Laboratory Animals’ (HHS publication 85-23, 1985, revised).

**Assay of serum T3 and T4**

Assay of serum T3 and T4 was carried out using \(^{125}\text{I}\)-labelled Coat-A-Count kit for T3 (TKT31) and T4 (TKT41) from Diagnostic Products Corporation (DPC). Trunk blood from the control \( (n = 6) \) and Tx rat dams \( (n = 5) \) was collected after birth. Trunk blood was also obtained at 120 d from control \( (n = 2; 1 \, \text{of each gender,}} \) representing 2 litters) and Tx offspring \( (n = 2; 1 \, \text{of each gender, representing 2 litters).} \)

**Emergence test**

In this test which has been described as a measure of emotional responsiveness (Attree et al., 1992) 8 controls and 8 Tx progeny (60- to 65-d-old) consisting of 4 females (representing 4 litters) and 4 males (representing
4 litters) were tested in 3 trials on consecutive days in the morning. The animal was removed from its cage and placed in a cylinder (diameter 15 cm x 20 cm length) which was positioned in the centre of a large Plexiglas box (90 cm x 90 cm x 45 cm). After opening a cardboard entrance flap, the emergence latency (with all four paws out) was recorded. The test was terminated at 5 min. In between testing of individual rats, the cylinder was washed and dried to eliminate effects on emergence performance due to remaining odours of the previously tested animal.

**Open-field activity**

Following emergence testing, the animals, described above for the emergence testing, were placed in the centre of the open field of the Plexiglas box (see above) and the number of lines crossed was recorded. The lines represented markings of 10 x 10 cm squares on the floor of the box. The total length of the test was 3 min. Open-field exploration was assessed once daily on 5 consecutive days. On the last 2 trials, emergence testing did not precede activity assessment. The floor of the Plexiglas box was cleaned between individual rat testing sessions.

**Lashley type 3 enclosed maze**

A separate group of rats was tested for their ability to learn a Lashley type 3 enclosed maze for a food reward (Munn, 1950). Rats, initially 80 d old, were tested on alternate days in 10 separate trials over a 20-d period. A total of 15 animals were used from each of the control (representing 6 litters) and Tx groups (representing 5 litters). Each treatment group consisted of 7 females and 8 males. Animals were tested in the maze once a day after food deprivation overnight. The maze was constructed by placing partitions (28 cm high) in the Plexiglas box described above. The maze consisted of four alleys, with food placed at the end cubicle opposite the starting point cubicle. In order to traverse the maze, the animal would have to make a right turn from the starting area, go down the first alley, make a left turn into the second alley, then turn right into the third alley, followed by a left turn into the fourth alley and a final right turn to get the food reward. The animals were marked for permanent identification, were randomized within treatment groups, and assessments conducted blind on each testing day. Each trial was terminated at 10 min. The time required to traverse the maze and the number of errors committed was recorded for each animal for each of the 10 trials. Errors consisted of wrong turns, retracing and going past the doors of the alleys. The floor of the maze was thoroughly cleaned between testing of individual animals.

**Biogenic amine/metabolite assay**

Brains were obtained from 55-d-old male rat progeny from control (n = 6, representing 4 litters) and Tx dams (n = 6 representing 4 litters) and the following brain regions dissected: cortex, hippocampus, olfactory tubercle (OT) and striatum. The tissues were weighed, frozen on dry ice and stored at -80 °C until use. The biogenic amines and major metabolites were determined by HPLC analysis as previously described (Davila et al., 1988). Briefly, tissues were sonicated in 0.1 N perchloric acid and the homogenates were microfuged for 8 min and filtered through a Micro-spin filter (USA Scientific Plastics). Diluted aliquots of the clear homogenate were mixed with an internal standard (3,4-dihydroxybenzylamine) and injected onto a reverse phase column (Supelcosil C-18 DB, Supelco). The mobile phase was pumped at 1 ml/min by a Spectra-Physics SP8800 programmable solvent delivery system. Electrochemical detection was performed with a Coulchem Model 5100 detector and triple electrode system. The output of the last electrode was recorded on a Spectra-Physics integrator. The regions assayed represent brain areas subserving some of the behaviours that may be disturbed in offspring of Tx dams. The cortex and hippocampus were selected based on their well-known role in cognition, learning and memory, and the striatum based on its established role in the maintenance of motor behaviours. The OT is of interest because this region, which contains relatively high concentrations of DA and 5-HT is an associative, sensorimotor component of the striatum. This structure is sensitive to THs during neuronal development (Clos and Legrand, 1990; Gottesfeld et al., 1987).

**Statistical analysis**

Data from the behaviour experiments were analysed by ANOVA using the Statistical Package for Social Sciences (SPSS) package and Tukey's t test for post-hoc comparisons. The HPLC data were analysed by ANOVA and post-hoc Student's t test using the STATVIEW program. Body-weight data were analysed using the Student's t statistic. Values of p less than 0.05 were considered statistically significant.

**Results**

**General health of progeny**

Throughout the experiment the progeny of Tx dams appeared to be healthy. Developmental milestones such as eye opening and righting reflex were not determined. Body weights were determined at birth, and at PN days 7, 21, 50 and 82. At birth the Tx progeny were significantly...
Cf, control females (n = 4); Tm, Tx males (n = 4); C, control females (n = 4); Txf, Tx females (n = 4). See text for litter representation. *p < 0.02 vs. control males. See text for overall comparisons.

lower in weight compared to control progeny [Tx (6.3 ± 0.5 g, n = 40) vs. controls (7.1 ± 0.9 g, n = 48), p < 0.001]. This difference was also seen at PN day 7 for the male [Tx (15.0 ± 1.4 g, n = 20) vs. controls (16.3 ± 1.8 g, n = 21), p = 0.018] and female progeny [Tx (13.5 ± 1.1 g, n = 20) vs. controls (14.2 ± 0.9 g, n = 27), p = 0.023]. At older ages, no significant differences were observed between Tx male and control male progeny or between Tx female and control female progeny (p ≥ 0.23). Although we did not determine the rate of weight gain during pregnancy, body weights of the Tx and control dams, determined on gestational day 20 were not significantly different (p = 0.376). Moreover no differences in gender breakdown of the litters were seen in offspring between control (males, 4.3 ± 1.7; females, 4.8 ± 1.8) and Tx litters (males, 4.2 ± 1.2; females, 3.8 ± 0.7).

**T4 and T3 levels**

Serum TH levels were decreased in Tx dams but were normal in their adult offspring. T4 levels in Tx dams (n = 5) were undetectable (< 1 µg/dl) while levels of T4 in controls were 52.5 ± 10.7 nM/l (n = 6). T3 levels were 3.5-fold lower in Tx dams (0.47 ± 0.14 nM/l) as compared to control dams (1.65 ± 0.28 nM/l). No significant differences in T4 or T3 levels were observed between the control (n = 2) and Tx progeny (n = 2) at 120 d (T4: control, 70.8 ± 5.5 nM/l vs. Tx, 65.6 ± 3.9 nM/l; T3: control, 1.69 ± 0.15 nM/l vs. Tx, 1.50 ± 0.20 nM/l).

**Emergence test**

Tx progeny emerged faster than control progeny (see Figure 1). ANOVA across all three consecutive trials demonstrated no significant differences within group trials (F2,36 = 2.019; p = 0.1381). Significant effects were seen with condition (F1,11 = 10.388; p = 0.012) and gender (F1,11 = 47.297; p = 0.0001). No significant interactions were seen with gender by trial, or condition by trial, however a significant interaction was observed between gender and condition (F1,11 = 9.406; p = 0.015). The significant condition effect appears to be due to the significantly shorter emergence times of the male Tx progeny (104.3 ± 29.3, p = 0.018) compared to the control males (225.3 ± 35.5). The emergence times of the Tx females were no different from the female controls (control progeny, 34.2 ± 19.3; Tx progeny, 31.1 ± 24.5, p = 0.924). Regardless of treatment, the females emerged significantly faster than the males (p < 0.01).

**Open-field activity**

The number of lines crossed in open-field exploration in the Tx and control progeny can be seen in Figure 2. No significant difference was seen across the five trials (F5,45 = 0.389; p = 0.815); however, significant gender (F1,6 = 57.092; p < 0.001) and condition effects (F1,6 = 4.004; p = 0.05) were seen. No significant gender by condition, gender by trial, or condition by trial interactions were observed. Although the progeny (males and females combined) of the Tx and control rat dams were more active than the control progeny, the difference was only marginally significant in males (control male progeny, 72.1 ± 9.59 vs. Tx male progeny, 97.45 ± 9.0, p = 0.062). Tx female progeny were only slightly more active than control females (controls, 148.6 ± 0.77 vs. Tx, 160.1 ± 8.95, p = 0.312). However, regardless of the treatment, female rats crossed significantly more lines than male progeny (p < 0.001).
The male control progeny took more than twice the amount of time to traverse the maze than the female progeny, irrespective of condition. However, neither control nor Tx progeny traversed the maze without committing errors, even in the tenth trial. ANOVA across all trials for errors made in maze learning also revealed a significant trial effect ($F_{9,11} = 2.923, p < 0.003$), a marginally significant group effect ($F_{1,11} = 2.920, p = 0.089$), but no significant gender ($F_{1,11} = 0.509, p = 0.476$) effect. However, a highly significant gender by condition interaction was observed ($F_{1,5} = 6.986; p = 0.009$). The female control and Tx progeny showed marginal improvement in the number of errors committed during the early trials ($p = 0.054$), while both the Tx males and control males committed a similar number of errors throughout trials 1–5 ($p > 0.70$). Analysis across trials 6–10 (see Figure 4) eliminated the significant trial differences in number of errors made. However, Tx progeny made significantly more errors than did control progeny ($F_{1,4} = 6.291; p = 0.013$). In the late trials, the gender by condition interaction was no longer evident ($p = 0.425$). Between early and late trials, the control progeny, irrespective of gender, and Tx female progeny showed significant reductions in the mean number of errors committed ($p < 0.03$), while the male Tx progeny failed to reduce the number of errors committed ($p > 0.90$). The better performance of the Tx females probably contributed significantly to the overall group by condition effect during trials 6–10, as the control females made significantly fewer errors than control males ($p = 0.003$); Tx males ($p < 0.0001$), or Tx females ($p = 0.008$). However, the Tx females performed as poorly as did the

**Lashley maze test**

Analysis of the time taken to transverse a maze across all trials revealed significant trial differences ($F_{9,11} = 7.53, p < 0.001$) and gender differences ($F_{1,11} = 44.683, p < 0.001$), but no significant group differences ($F_{1,11} = 0.574, p = 0.450$). In agreement with a previous study (Hendrich et al., 1984) real learning did not become apparent with most of the progeny until trial 6. The male control progeny slowly improved their performance from trials 2–5 ($p < 0.05$), while the performance of the Tx male progeny was similar throughout trials 1–5 ($p = 0.839$). The control females improved their performance from one trial to the next in trials 1–5 ($p < 0.02$), however significant improvement in the Tx females was only seen between trials 1 and 5 ($p < 0.05$). With the sixth trial, both male and female control animals showed a dramatic improvement in performance, whereas the performance of the male and female Tx progeny did not significantly improve. During trials 6–10, no significant trial differences were seen in time to traverse the maze ($F_{4,6} = 0.842, p = 0.439$), suggesting that maze learning had stabilized and the Tx progeny showed significantly longer maze running times as compared to control progeny ($F_{1,4} = 4.241, p = 0.047$) (see Figure 3). Further analysis across trials 6–10 revealed a marginally significant gender by condition interaction ($F_{1,4} = 3.830, p = 0.058$) but no gender by trial ($F_{1,4} = 0.497, p = 0.612$) or group by trial ($F_{1,4} = 0.322, p = 0.727$) interactions. The difference appears to be largely due to the overall better maze running time of both control and Tx females. The males took more than
Table 1. Biogenic amine and metabolite levels in control and Tx progeny

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>DA (pg/g)</th>
<th>HVA (pg/g)</th>
<th>DOPAC (pg/g)</th>
<th>NE (pg/g)</th>
<th>5-HT (pg/g)</th>
<th>5-HIAA (pg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT</td>
<td>Control</td>
<td>5.37 ± 1.97</td>
<td>1.09 ± 0.24</td>
<td>2.67 ± 1.10</td>
<td>0.69 ± 0.25</td>
<td>2.51 ± 1.09</td>
<td>5.94 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>Tx</td>
<td>3.89 ± 1.08</td>
<td>0.57 ± 0.11**</td>
<td>2.09 ± 0.80†</td>
<td>0.55 ± 0.21</td>
<td>1.71 ± 0.54</td>
<td>2.74 ± 0.88*</td>
</tr>
<tr>
<td>Cortex</td>
<td>Control</td>
<td>0.13 ± 0.06</td>
<td>0.09 ± 0.03</td>
<td>0.09 ± 0.05</td>
<td>0.29 ± 0.05</td>
<td>0.25 ± 0.03</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Tx</td>
<td>0.14 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.25 ± 0.08</td>
<td>0.22 ± 0.02**</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Control</td>
<td>0.05 ± 0.04†</td>
<td>0.08 ± 0.06†</td>
<td>0.06 ± 0.05</td>
<td>0.52 ± 0.18</td>
<td>0.51 ± 0.17</td>
<td>0.63 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Tx</td>
<td>0.05 ± 0.03†</td>
<td>0.37 ± 0.08**</td>
<td>0.02 ± 0.01</td>
<td>0.50 ± 0.07</td>
<td>0.44 ± 0.08</td>
<td>0.89 ± 0.26</td>
</tr>
<tr>
<td>Caudate</td>
<td>Control</td>
<td>5.34 ± 0.16†</td>
<td>1.04 ± 0.27†</td>
<td>1.33 ± 0.47†</td>
<td>0.58 ± 0.15†</td>
<td>0.45 ± 0.11†</td>
<td>0.49 ± 0.14‡</td>
</tr>
</tbody>
</table>
|            | Tx        | 5.04 ± 1.02 | 1.04 ± 0.23 | 1.15 ± 0.24 | 0.44 ± 0.11 | 0.35 ± 0.04 | 0.44 ± 0.04   

*p < 0.05, **p < 0.01 vs. control. Values are mean ± s.d. (n = 6) or † n = 5 (missing samples) in pg/g of tissue.

Tx males (p = 0.111). The increased activity of the Tx progeny may also have contributed to their larger number of errors and erratic performances. They appeared hurried, passed gates and had to retrace their paths.

Biogenic amine levels

HPLC analyses of the levels of dopamine (DA) and its major metabolites, DOPAC and HVA; norepinephrine (NE); serotonin (5-HT) and its metabolite 5-HIAA; were carried out on 53-d-old male control and male Tx progeny. As can be seen in Table 1, NE metabolism was not significantly altered by maternal hypothyroidism in any of the regions examined. DA was reduced by 34% in the OT of Tx progeny but the difference was not significant (p = 0.14). In the OT, the Tx progeny had significantly lower levels of the DA metabolite, HVA (p = 0.0008), while reductions in DA and DOPAC levels were not significantly different from control offspring (p = 0.14, p = 0.30, respectively). In contrast, in the hippocampus, significantly higher levels of the DA metabolite HVA (p = 0.0003) were found in Tx progeny while DA and DOPAC levels were reduced by 15% (p = 0.75) and 58% (p = 0.14), respectively. Serotonin metabolism was also altered in the OT and cortex of Tx progeny. In the OT, 5-HIAA levels were 46% of control progeny levels (p = 0.03); however 5-HT levels, 31% lower in the Tx rats, were not significantly different from control progeny (p = 0.14). Cortical 5-HIAA levels were also significantly reduced in the Tx progeny (p = 0.006), but 5-HT levels remained unchanged (p > 0.90).

Discussion

The irreversible and deleterious neurological effects of an inadequate supply of THs during development have been known for some time. The earlier view that THs of maternal origin were not of significant importance for fetal brain development (Fisher et al., 1977) was based on inadequate knowledge of maternal–fetal placental transport of T4 and T3. This consensus resulted in studies focused primarily on the effects of fetal TH deficiency. Thus, most of the experimental evidence on the adverse neurodevelopmental consequences of thyroid deficiency derived from studies utilizing iodine-deficient or chemical thyroidectomy (e.g. methimazole) models. These animal models result in maternal, fetal and neonatal hypothyroxinaemia and therefore do not mimic the thyroid state of some pregnant women with hypothyroxinaemia. Despite the insight provided by these earlier studies, few studies have assessed whether deprivation of T4 of maternal origin in the euthyroid fetus in early fetal life results in detrimental effects on cognitive and neurological function, although there is now ample evidence that an adequate, though limited, supply of maternal T4 is available early in fetal development through placental transport (Ekins et al., 1994; Obregon et al., 1984; Porterfield and Hendrich, 1992).

The results of our study using the Tx rat dam model, with cross-fostering of offspring to untreated dams, and without T4 treatment of the Tx rat dams before conception, support the hypothesis that fetal deprivation of THs prior to fetal thyroid hormonogenesis results in neurobehavioural dysfunction and also produces disturbances in the metabolism of DA and 5-HT in specific brain regions. Our findings are similar to previous studies in some regards, but also different in others.

Emergence latencies into an open field with subsequent activity level assessments were previously determined in progeny of Tx rat dams (Attree et al., 1992). This report showed that progeny of Tx rat dams had longer emergence latencies than did control animals and significant gender differences were not found. In our study, the emergence latencies were shorter in Tx progeny than in controls, an effect contributed mainly by Tx males as the Tx females had emergence times similar to control females. In the study of Attree et al. (1992) the Tx
progeny were cross-fostered, but it is unclear whether a separate group of untreated nursing dams was used. Also, we utilized a cylinder for emergence testing, which was smaller and longer than the rectangular box utilized in the previous study. Our test also required that all four paws of the rat be out of the cylinder.

The activity levels determined directly after emergence testing were higher in the Tx progeny in the present study, irrespective of gender. This finding is consistent with the findings of Hendrich et al. (1984), while the exploratory behaviour seen in Tx progeny following emergence testing was reduced in another study (Attree et al., 1992). The difference in the latter study was apparently due to the higher activity levels of the control females in comparison to all other groups. In the present study, higher activity levels of the control females were also a contributor to the overall difference seen between Tx and control progeny. However, the Tx female progeny were more active than all of the other groups and while the males from control and Tx dams were less active than either female group, the Tx males had higher activity levels than the control males. This increased activity in the Tx progeny may have been a factor in the less cautious behaviour exhibited by these progeny in emergence testing. The differences in the results of our study with those of Attree et al., (1992) may reflect variable effects of maternal TH deficiency (see below).

The present study also showed that learning in Tx progeny is compromised and also that learning behaviour differs in male and female progeny of Tx dams. Even though the Tx females learned the maze faster and made fewer errors than did the male Tx progeny or control male progeny, the degree of impairment of the Tx females relative to their control counterparts appeared to be more severe. Interestingly, the maze performance of the control males was equivalent to that of the Tx females and only slightly better than the learning exhibited by their Tx male counterparts. Our findings are similar to those of Hendrich et al. (1984), even though the Tx dams in that study received T4 treatment prior to mating and also during nursing of their Tx progeny.

Previously, the influence of THs on brain neurotransmitters has only been investigated in neonatal rats chemically thyroidectomized at birth and the results have been inconsistent (Dupont et al., 1981; Rastogi et al., 1976). Utilizing the Tx rat dam model which focuses on the fetal effects of maternal T4 deficiency, more recent studies have suggested an association between neurotransmitter dysfunction and the behavioural impairments observed in Tx progeny (Evans et al., 1999; Hendrich et al., 1984; Sinha et al., 1994). Evans et al. (1999) found that the activities of monoamine oxidase (MAO), tyrosine hydroxylase, dopa-decarboxylase and choline acetyl-transferase (ChAT) were elevated in cortex of 10- to 20-d-old rats born to Tx dams, but not in 30-d-old animals. However, an earlier report showed that MAO and ChAT activities are compromised in adult progeny of Tx dams (Sinha et al., 1994), suggesting that some disturbances may become apparent over time. The region-specific changes in the metabolism of DA and 5-HT in the brains of adult male progeny of Tx dams seen in the present study, are the first data showing possible fetal effects of maternal thyroid deficiency on biogenic amine function. The decrease in the OT in DA, HVA and DOPAC, and 5-HT and 5-HIAA may reflect decreased synthesis and or increased degradation. Inasmuch as olfactory cues play a role in learning processes, the alterations seen in DA and 5-HT metabolism may be indirectly associated with the learning deficits seen in the Tx progeny. Cortical 5-HIAA levels were also decreased in Tx progeny while 5-HT levels were similar, which may indicate increased degradation and or decreased synthesis. Although limited, these data may be associated with the learning impairments seen in the Tx progeny, inasmuch as there are reports showing an association between learning deficits and reduced cortical serotonergic activity (Stemmlein et al., 2000). In contrast to the OT, hippocampal HVA levels were increased in the Tx progeny; however, DA and DOPAC levels were not significantly affected. Although the effect of maternal hypothyroxinaemia on dopaminergic activity in the hippocampus is not clear from these limited data, our findings are consistent with reports indicating that DA receptor-mediated activity plays an important role in regulating learning and memory. Dopamine D2 receptors appear to facilitate (Sigala et al., 1997), while D1 and D3 receptors appear to inhibit learning and memory (Izquierdo et al., 1998; Sigala et al., 1997). It is well established that the biogenic amine transmitter systems follow a differential and region-specific developmental course in postnatal life. Therefore, the influence that in utero maternal TH deficiency may have on the developmental profile and functioning (e.g. changes in DA and 5-HT receptor sensitivities) of these neurotransmitters is unclear. A sufficient number of offspring were not available to examine the metabolism of the neurotransmitters at earlier and later ages, or in female progeny.

The roles of THs in fetal development are complex and not completely understood. Inconsistencies in our results with other findings suggest that a number of parameters may influence the effects seen in Tx progeny. It is possible that certain behaviours of the thyroid-deficient dam may have compromised nutritional status, activity level, and other physiological and psychological functions and therefore indirectly influenced our results. The degree of thyroid deficiency in the rat dams may also be a factor. T4 and T3 have been detected in brains of fetuses from
control dams as early as gestation day 13, while brains from fetuses of Tx dams contain lower T4 and T3 levels both prior to and after the development of fetal thyroid function (Porterfield and Hendrich, 1992). Moreover, there is some evidence that there is net flux of TH from the fetus to the Tx rat dam in late gestation (Porterfield and Hendrich, 1993) which may transiently affect fetal thyroid economy. We found that the Tx dams at parturition had no measurable T4 and significantly less T3 than control dams, but we did not determine the TH status in serum or brain of the Tx offspring in early PN life. Serum T4 and T3 levels in our adult Tx progeny were similar to control levels.

From the available evidence and the results described in this report, it would appear that sufficient TH is available through placental transport early in fetal development and may protect the euthyroid fetus in utero from the neurodevelopmental insults resulting from maternal thyroid deficiency. Neurobehavioural compromise in such progeny is significant, and from the report of Hendrich et al. (1984) it appears that neonatal exposure of Tx progeny to maternal T4 does not correct the damage. Replacement T4 treatment during fetal life has been suggested to reduce some of the adverse effects of chemically induced maternal and fetal hypothyroxinaemia (Weller et al., 1996); however this has not been tested in the Tx rat model where the fetus is euthyroid. While it is clear from studies of fetal hypothyroidism that THs are required for normal fetal neurodevelopment, the results in this report emphasize that making sufficient amounts of THs available to the euthyroid fetus may minimize the impact of maternal TH deficiency in utero. Identifying the optimum amount of T4 supplementation and optimum time for T4 administration during gestation in the Tx rat model may clarify the usefulness of replacement T4 therapy in some pregnant women.

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References


Euthyroid fetuses and maternal hypothyroxaemia


