



## Comparative study of the effects of experimentally induced hypothyroidism and hyperthyroidism in some brain regions in albino rats

A.M. El-bakry<sup>a,\*</sup>, A.W. El-Gareib<sup>b</sup>, R.G. Ahmed<sup>a</sup>

<sup>a</sup> Zoology Department, Faculty of Science, Beni Suef University, Egypt

<sup>b</sup> Zoology Department, Faculty of Science, Cairo University, Egypt

### ARTICLE INFO

#### Article history:

Received 3 January 2010

Received in revised form 7 April 2010

Accepted 9 April 2010

#### Keywords:

Thyroid states

Development

Brain

Skeleton

### ABSTRACT

Thyroid hormones (THs) play a crucial role in the development and physiological functioning of different body organs especially the brain. Therefore, the objective of this study was to show the histopathological effects of the different thyroid states on some brain regions (cerebrum and cerebellum) and the skeletal features of their newborns during the postnatal development from the 1st to 3rd week. The female white albino rats were allocated into 3 groups as follows: the experimental hypothyroidism group is induced by 0.02% methimazole (MMI) (w/v) in drinking water, while the experimental hyperthyroidism group is performed by exogenous T4 [from 50 to 200 µg/kg body weight intragastric administration beside adding 0.002% T4 (w/v) to the drinking water] from the gestation day 1 to lactation day 21 and control group which received tap water.

As well, both maternal hypo- and hyperthyroidism caused some malformation and developmental defects in the cerebellar and cerebral cortex of their newborns along the duration of the experiment. These degenerative symptoms became more prominent and widely spread at the 3rd postnatal week. Concomitantly, there were some degeneration, deformation and severe growth retardation in neurons of these regions in both treated groups throughout the experimental period. Moreover, the skeletal features of these newborns were accelerated in hyperthyroid group while these maturations were delayed partially in hypothyroid ones during the examined periods. These alterations, on both treated groups, were age and dose dependent. Thus, further studies need to be done to emphasize this concept.

Published by Elsevier Ltd on behalf of ISDN

### 1. Introduction

In the last fifteen years, an increasing number of studies have indicated that thyroid hormones (THs) [thyroxine (T4) and tri-iodothyronine (T3)] have important physiological functions, not only during brain maturation but also in the adult vertebrate brain (Broedel et al., 2003; Horn and Heuer, 2010). Several reports have been published on the essential role of the THs for mammalian and non-mammalian brain development (Bruno et al., 2005; Gilbert and Sui, 2006; Carageorgiou et al., 2007; Ahmed et al., 2008b). More importantly, the best-defined animal model of thyroid hormone-

dependent brain development is the neonatal rat (Legrand, 1986; Schwartz et al., 1997).

Pertaining to the normal neurological development, the THs act in the following manner (Porterfield, 1994): (a) they increase the rate of neuronal proliferation in the cerebellum (Dussault and Ruel, 1987), (b) they act as the “time clock” to end neuronal proliferation and stimulate differentiation (Dussault and Ruel, 1987; Pasquini and Adamo, 1994), (c) once neurons are formed, they follow an orderly pattern of migration to the appropriate regions in the brain, particularly, in the cerebral and cerebellar cortex (Süha and Çalikoğlu, 1999), (d) they stimulate the formation and development of neuronal processes, axons and dendrites, (Klein et al., 1972) and (e) development of neuronal processes leads to formation and development of synapses.

Several reports are listed on the harmful effect of thyroid hormone deficiency during the development (Ahmed et al., 2008b; Hasebe et al., 2008; Zhang et al., 2010). The previous publications have demonstrated that the thyroid deficiency produces: Deficient cellular maturation in the cerebral cortex of hypothyroid rats (Schwartz et al., 1997), Altered molecular, morphological and functional in cerebral cortex during development (Lee et al., 2003) and makes defects in the myelination of the cerebral cortex and the

**Abbreviations:** BV, blood vessel; b. wt., body weight; CB, cerebellum; DC, degenerative changes; EGC, external granular cells; EGL, external granular layer; HBV, hyperemic blood vessel; HP, hyperplastic proliferation; Hy, hyperemia; Hyp, hyperplasia; IGL, internal granular layer; ML, molecular layer; NMP, neutral mucopolysaccharides; O, oedema; PC, Purkinje cell; PCO, pericellular oedema; PVO, perivascular oedema; PYC, pyramidal cells; TEGL, thickened external granular layer; TM, thickened meninges; V, vacuoles; VDC, vacuolar degenerative changes; WM, white matter; w/v, weight per volume.

\* Corresponding author. Tel.: +02 082 5822423.

E-mail address: [amalbakry2@yahoo.com](mailto:amalbakry2@yahoo.com) (A.M. El-bakry).

maturity of neuronal circuits leading to permanent brain dysfunction (Wong and Leung, 2001). Taken together, altered thyroid status disturbed the maturation of the CB and led to defects in granule cell migration, PC arborization, the timing of apoptosis, and neuronal integration (Legrand, 1967a,b). Overall, a deficiency of THs in the neonatal rat has been shown to cause disorganization of the cerebellar cortex (Nicholson and Altman, 1972b,c; Nunez, 1984). More so, a reduction, or absence, of thyroid hormone during brain maturation yields molecular, morphological and functional alterations in the CB (Lee et al., 2003; Bhanja and Chainy, 2010).

On other words, the early hyperthyroidism in rats alters thyroid states and caused some malformations as decrease in body, brain and cerebellar weight (Lauder, 1977; Nicholson and Altman, 1972a). Generally, hyperthyroid animals appear to have a shorter life and, at advanced age, show a myelin deficiency (Pasquini and Adamo, 1994).

Generally, one of the most pronounced actions of THs is to influence growth, differentiation and development of skeletal tissues (cartilages and bones) during pre- and postnatal life (Awad, 2002; O'Shea et al., 2003; Kvistad et al., 2004; Kosińska et al., 2005). THs in human are necessary for normal bone growth (Kvistad et al., 2004) via both direct effects on bone cells and indirect effects by stimulation of growth hormone release. In addition, Sbaihi et al. (2007) demonstrated that THs may participate in the mobilization of bone mineral stores in the eel, by inducing different types of vertebral bone resorption. On the other hand, childhood hypothyroidism results in growth arrest, delayed bone age, epiphyseal dysgenesis and short stature (Chiesa et al., 1994). Freitas et al. (2005) reported that hypothyroidism impaired longitudinal body growth and delayed ossification in rat. In hyperthyroidism, the skeletal growth and maturation was increased (Segni and Gorman, 2001; Kosińska et al., 2005). Also, the elevation of calcium under the effect of thyroxine may result from the effect of this hormone on an increasing bone turnover (Hoch, 1974). Finally, this work can offer an important contribution through establishing some specific basis for the postnatal interactions between the THs and brain development of rat newborns. Concurrently, the present study aimed to assess the effect of hypothyroidism and hyperthyroidism in various brain regions (cerebellar cortex, cerebellum) of rat newborns and their skeletal features during the postnatal development.

## 2. Materials and methods

### 2.1. Experimental animals

White albino rats from order rodentia and family muridae were used in this experiment. The present study was carried out on 69 albino rat, 46 mature virgin females weighting about 170–190 g (g) and 23 mature males. They were obtained from the National Institute of Ophthalmology, Giza, Egypt. The adult rats were kept under observation in the department animal house for 2 weeks to exclude any intercurrent infection and to acclimatize the new conditions. The culled animals were marked, housed in metal (stainless steel) separate bottom good aerated cages at normal atmospheric temperature ( $23 \pm 2^{\circ}\text{C}$ ) and fed on standard rodent pellet diet manufactured by the Egyptian Company for oil and soap as well as some vegetables as a source of vitamins. Tap water was used for drinking *ad libitum* and these animals were maintained at constant daily light/dark periods of 12 h each (lights on at 06:00 h) and  $50 \pm 5\%$  relative humidity. All animal procedures are in accordance with the general guidelines of animal care and the recommendations of the Canadian Committee for Care and use of animals (Canadian Council on Animal Care, 1993). All efforts were made to minimize the number of animals used and their suffering. Mating was induced by housing prooestrous females with male in separate cage at ratio of two females and one male overnight for one or two consecutive days. In the next morning, the presence of sperm in vaginal smear determined the first day of gestation. Then, the pregnant females were transferred into separate cages from males to start the experiment.

### 2.2. Experimental schedule

The adult female rats from the 1st day of pregnancy [gestation day (GD) 1] to the first 3 weeks of lactation period [lactation day (LD) 21] were allocated into three groups as follows:

(1) *Hypothyroid group*: Fifteen rats were rendered hypothyroid by administration of antithyroid agent, methimazole (MMI) (Sigma Chemical Company, USA), an inhibitor of triiodothyronine (T3) and thyroxine (T4) synthesis (Ornellas et al., 2003; Hasebe et al., 2008), in drinking water at concentration of 0.02% (weight per volume; w/v) (Venditti et al., 1997) directly after mating (GD 1–LD 21).

(2) *Hyperthyroid group*: Further fifteen rats were rendered hyperthyroid by exogenous thyroxine (T4) (Eltroxine tablets; GlaxoWellcome, Germany) intragastric administration in increasing doses beginning from 50 to reach 200 µg/kg body weight (b. wt.) beside adding 0.002% (w/v) T4 to the drinking water (Guerrero et al., 1999; Abdel-Moneim, 2005; Ahmed, 2006) directly after mating (GD 1–LD 21).

(3) *Control group*: Sixteen control rats received tap water.

The mother sera (6 per group) were taken during the pregnancy at day 10 and after pregnancy at day 10 to estimate the total triiodothyronine (TT3) and total thyroxine (TT4) in normal, hypothyroid and hyperthyroid status. The blood samples were taken from optic vein and centrifuged at 3000 round per minute (r.p.m.) for 30 minutes (min). The clear, non-hemolysed supernatant sera were quickly removed, divided into three portions for each individual animal, and kept at  $-30^{\circ}\text{C}$  until use.

After the pregnancy, the decapitation of normal, hypothyroid and hyperthyroid newborns was done at the end of the 1st, 2nd and 3rd postnatal weeks under mild diethyl ether anaesthesia. The brain was finally separated from the skull base after cutting all the cranial nerves and divided into cerebrum and cerebellum. Samples from brain regions were immediately fixed in 20% formal saline solution then stored in 70% alcohol for a general histological preparation.

### 2.2.1. Estimation of serum T4, T3, TSH and GH levels

Estimation of total thyroxine (TT4), total triiodothyronine (TT3) in sera of mothers and free thyroxine (FT4), free triiodothyronine (FT3), thyrotropin (TSH) and growth hormone (GH) in sera of their newborns were determined in Diabetic Endocrine Metabolic Pediatric Unit, Center for Social and Preventive Medicine, New Children Hospital, Faculty of Medicine, Cairo University according to the methods of Thakur et al. (1997), Maes et al. (1997), Larsen (1982), Smals et al. (1981), Mandel et al. (1993) and Reutens (1995), respectively. The kits for these hormones were obtained from Calbiotech INC (CBI), USA.

### 2.2.2. Haematoxylin and eosin stain (Bancroft and Stevens, 1982)

The investigated regions (cerebellum and cerebrum) of each group were dehydrated in ascending series of ethyl alcohol then placed in xylene for clearance. The tissues were placed in paraffin wax and cut serially at 5 µm thickness. These sections were de-waxed, hydrated and stained in Mayer's haemalum solution for 3 min. These sections were stained in eosin for one minute. The sections were washed in running tap water and dehydrated in ascending series of ethyl alcohol. Clearing in xylene and mounting in Canada balsam were done.

### 2.2.3. Periodic acid Schiff (PAS) reaction stain (Bancroft and Stevens, 1982)

The prepared serial sections of cerebellum and cerebrum were de-waxed then hydrated. These sections were oxidized in 1% aqueous periodic acid for 5 min, then washed in running water for 5 min and rinsed in diluted water. They were placed in Schiff's reagent for 10–20 min, then washed for 30 min in running water and rinsed three times in 0.5% aqueous sodium metabisulphite, freshly prepared and washed in running water for 10 min. Nuclei were stained in Celestine blue-haemalum sequence, differentiated in acid alcohol and bleached in running water. Thereafter, the sections were dehydrated in alcohol, cleared in xylene and mounted in Canada balsam. However, production of standard serial sections was technically difficult because the brain must be sectioned at a consistent level and the cuts must be made truly vertical with no oblique angles in any of the axes (Duffell et al., 2000).

### 2.2.4. Skeletal features of rat newborns

The study followed the body length, as a general gross morphology, in control, hypothyroid and hyperthyroid groups of rat newborns at the end of the 1st, 2nd and 3rd weeks of birth. Moreover, the general skeletal features of rat newborns were noticed by the X-ray examination (6 ml ampere and 40 kvolt/0.5 s). The C.R. X-ray system purchased from Voge Company, Japan, 1990.

### 2.3. Statistical analysis

The data are analyzed using one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985) followed by LSD analysis to discern the main effects and compare various groups with each other. F-probability for each variable expresses the general effect between groups. A two-way analysis of variance was also applied to evaluate the effect of age, treatment and their interaction during the experimental period. The data are presented as mean  $\pm$  standard error (SE) and values of  $P > 0.05$  are considered statistically non-significant while those of  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  are considered statistically significant, highly significant and very highly significant, respectively.

**Table 1**

Effect of thyroid status on total thyroxine (TT4,  $\mu\text{g}/100\text{ ml}$ ) and total triiodothyronine (TT3,  $\text{ng}/100\text{ ml}$ ) concentrations in serum of pregnant rats.

Hormones Status	TT4	TT3
At day 10 during pregnancy		
Control	4.495 $\pm$ 0.137 <sup>c</sup>	77.501 $\pm$ 0.670 <sup>d</sup>
Hypothyroid	2.745 $\pm$ 0.039 <sup>d</sup>	31.001 $\pm$ 0.894 <sup>e</sup>
Hyperthyroid	5.250 $\pm$ 0.068 <sup>b</sup>	90.000 $\pm$ 0.894 <sup>b</sup>
At day 10 after postpartum		
Control	5.535 $\pm$ 0.060 <sup>b</sup>	87.050 $\pm$ 4.896 <sup>b</sup>
Hypothyroid	1.600 $\pm$ 0.084 <sup>e</sup>	13.501 $\pm$ 0.670 <sup>f</sup>
Hyperthyroid	10.450 $\pm$ 0.335 <sup>a</sup>	129.003 $\pm$ 5.021 <sup>a</sup>
LSD at 5% level	0.289	1.900
LSD at 1% level	0.389	2.560
F-probability	$P < 0.001$	$P < 0.001$

- Data are expressed as mean  $\pm$  SE. Number of animals in each group is six.

- For each parameter, values which share the same superscript symbols are not significantly different.

- F-probability expresses the effect between groups, where  $P < 0.001$  is very highly significant.

### 3. Results

#### 3.1. Serum-hormonal levels (thyroid function): (Tables 1–3)

The present work comprised the disturbance induced in serum-hormonal system of pregnant rats and their newborns in response to administrations of methimazole (MMI) and thyroxine (T4) to mothers from gestation day (GD) 1 to lactation day (LD) 21.

Table 1 showed that higher serum TT4 (Maternal total thyroxine) and TT3 (total triiodothyronine) of adult female rats at day 10

postpartum than those at gestational day 10 in control group. TT4 and TT3 levels were significantly lower (LSD;  $P < 0.01$ ) in hypothyroid rats than in controls and remained lower at day 10 after the birth but in hyperthyroid rat the serum TT4 and TT3 levels increased significantly (LSD;  $P < 0.01$ ) at day 10 during pregnancy and after birth.

In table (2), the concentrations of FT4 (Free thyroxine), FT3 (free triiodothyronine), TSH (thyrotropin) and GH (growth hormone) in normal rat newborns were increased with the age progress in all investigated periods. At all testing periods, the baseline levels of serum FT4, FT3 and GH were decreased significantly (LSD;  $P < 0.01$ ) below normal values in newborns of hypothyroid mothers whose serum TSH levels were significantly elevated (LSD;  $P < 0.01$ ). However, FT4, FT3 and GH levels in newborns of hyperthyroid mothers were increased significantly (LSD;  $P < 0.01$ ); their serum TSH levels were significantly lower (LSD;  $P < 0.01$ ) as the age progressed from the 1st to 3rd postnatal weeks as compared with the corresponding controls (Table 2). Moreover, at the end of the 3rd week, TSH levels in hyperthyroid group were very low as compared to the levels in the age-matched normal controls ( $0.150 \pm 0.022$  vs.  $1.750 \pm 0.021$ ).

With regards one-way ANOVA of TT4, TT3, FT4, FT3, TSH and GH in table (3), the general effect between groups was found to be very highly significant ( $P < 0.001$ ) throughout the experiment. Furthermore, two-way analysis of variance of TT4 verified that the effect of age, hyperthyroidism and their interaction was very highly significant on and a similar pattern was also observed in hypothyroid group except for the effect of age which was non-significant ( $P > 0.05$ ). On the other hand, the effect of hyperthyroidism on TT3 was very highly significant while the effect of age alone was significant ( $P < 0.05$ ) and their interaction was highly significant ( $P < 0.01$ ). As the effect of hypothyroidism on TT3 was very highly signifi-

**Table 2**

Effect of thyroid status on free thyroxine (FT4,  $\text{ng}/100\text{ ml}$ ), free triiodothyronine (FT3,  $\text{pg}/100\text{ ml}$ ), thyrotropin (TSH,  $\text{ng}/100\text{ ml}$ ) and growth hormone (GH,  $\text{ng}/100\text{ ml}$ ) concentrations in serum of rat newborns.

Periods	Hormones Status	FT4	FT3	TSH	GH
1 Week	Control	2.801 $\pm$ 0.044 <sup>f</sup>	42.501 $\pm$ 1.119 <sup>f</sup>	0.951 $\pm$ 0.022 <sup>f</sup>	1.750 $\pm$ 0.067 <sup>f</sup>
	Hypothyroid	1.950 $\pm$ 0.021 <sup>g</sup>	36.504 $\pm$ 0.670 <sup>g</sup>	2.451 $\pm$ 0.067 <sup>c</sup>	0.950 $\pm$ 0.022 <sup>g</sup>
	Hyperthyroid	3.351 $\pm$ 0.067 <sup>d</sup>	50.503 $\pm$ 0.670 <sup>e</sup>	0.701 $\pm$ 0.044 <sup>g</sup>	1.950 $\pm$ 0.021 <sup>e</sup>
2 Week	Control	3.159 $\pm$ 0.067 <sup>e</sup>	54.500 $\pm$ 1.119 <sup>d</sup>	1.350 $\pm$ 0.068 <sup>e</sup>	2.551 $\pm$ 0.022 <sup>d</sup>
	Hypothyroid	1.450 $\pm$ 0.066 <sup>h</sup>	27.008 $\pm$ 0.894 <sup>h</sup>	3.751 $\pm$ 0.068 <sup>b</sup>	0.750 $\pm$ 0.021 <sup>h</sup>
	Hyperthyroid	3.801 $\pm$ 0.044 <sup>b</sup>	59.507 $\pm$ 0.223 <sup>c</sup>	0.401 $\pm$ 0.044 <sup>h</sup>	2.751 $\pm$ 0.023 <sup>c</sup>
3 Week	Control	3.550 $\pm$ 0.022 <sup>c</sup>	66.001 $\pm$ 1.341 <sup>b</sup>	1.750 $\pm$ 0.021 <sup>d</sup>	3.301 $\pm$ 0.044 <sup>b</sup>
	Hypothyroid	0.551 $\pm$ 0.022 <sup>i</sup>	19.000 $\pm$ 0.670 <sup>e</sup>	4.051 $\pm$ 0.021 <sup>a</sup>	0.501 $\pm$ 0.045 <sup>i</sup>
	Hyperthyroid	4.150 $\pm$ 0.067 <sup>a</sup>	69.501 $\pm$ 0.670 <sup>a</sup>	0.150 $\pm$ 0.022 <sup>i</sup>	3.652 $\pm$ 0.021 <sup>a</sup>
LSD at 5% level		0.148	2.492	0.134	0.103
LSD at 1% level		0.198	3.355	0.181	0.139

- Data are expressed as mean  $\pm$  SE. Number of animals in each group is six.

- For each variable, values which share the same superscript symbols are not significantly different.

- F-probability expresses the effect between groups, where  $P < 0.001$  is very highly significant.

**Table 3**

Two-way analysis of variance (ANOVA) for TT4 and TT3 concentrations in serum of pregnant rats and for FT4, FT3, TSH and GH concentrations in serum of their newborns.

Source of Variation	F-probability					
	TT4	TT3	FT4	FT3	TSH	GH
(1.) Control. Hypothyroid						
- General effect	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
- Hypothyroid	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
- Age	$P > 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$
- Hypothyroid X Age	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
(2.) Control. Hyperthyroid						
- General effect	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
- Hyperthyroid	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P > 0.05$
- Age	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.001$
- Hyperthyroid X Age	$P < 0.001$	$P < 0.01$	$P > 0.05$	$P > 0.05$	$P < 0.001$	$P > 0.05$

$P > 0.05$  is non-significant,  $P < 0.05$  is significant,  $P < 0.01$  is highly significant and  $P < 0.001$  is very highly significant.

cant, the effect of age and their interaction was highly significant. The effect of hypothyroidism and its interaction with age on FT4, FT3, TSH and GH was very highly significant. The effect of age of hypothyroid newborns alone was significant ( $P < 0.05$ ) on FT3 level while it was very highly significant on FT4 and TSH and on GH level. On the other hand, the effect of hyperthyroidism and age was very highly significant on FT4 and FT3 levels but the effect of their interaction was non-significant ( $P > 0.05$ ). Also, the effect of hyperthyroidism and its interaction with age on TSH level was very highly significant and on GH level was non-significant ( $P > 0.05$ ) while the effect of age alone was significant on TSH and very highly significant on GH (Table 3).

### 3.2. The cerebellar cortex: (Figs. 1–4)

#### 3.2.1. General histology (haematoxylin and eosin stain): (Figs. 1–3)

**3.2.1.1. In normal group.** The external granular layer (EGL) was found to be the outer layer of the cortex and their cells were difficult to see clearly (Figs. 1A<sub>1</sub>, 2A<sub>1</sub>, and 3A<sub>1</sub>). Furthermore, the molecular layer (ML) was shown located between the EGL externally and the Purkinje cell layer internally (Figs. 1A<sub>1</sub>, 2A<sub>1</sub>, and 3A<sub>1</sub>). Moreover, it was found that the Purkinje layer (PL) comprised pear-shaped neurons and was arranged in rows at a junction of the ML and internal granular layer (IGL); in a single row at the end of the 1st postnatal week (Fig. 1A<sub>2</sub>), in two rows at the end of the 2nd postnatal week (Fig. 2A<sub>2</sub>) and in three rows at the end of the 3rd postnatal week (Fig. 3A<sub>2</sub>). A large rounded nucleus was evident in each cell body of the Purkinje cells (PCs) with thick remarked cytoplasmic coat from the end of the 1st to 3rd weeks old (Figs. 1A<sub>2</sub>, 2A<sub>2</sub>, and 3A<sub>2</sub>). Also, the IGL was shown situated just below the Purkinje cell layer (PL) and it was extremely cellular and formed of closely packed oval or rounded shaped neurons with the age progress (Figs. 1A<sub>1</sub> and A<sub>2</sub>, 2A<sub>1</sub> and A<sub>2</sub>, and 3A<sub>1</sub> and A<sub>2</sub>).

**3.2.1.2. In hypothyroid group.** At the end of the 1st postnatal week, there were oedematous areas observed in the ML and IGL (Fig. 1B<sub>4</sub>) associated with some reduction in the size of the ML (Fig. 1B<sub>2</sub>–B<sub>4</sub>). There were hemorrhagic areas as hyperemic blood vessel between the folds and fissures (Fig. 1B<sub>3</sub>) and also hyperemia in the IGL were observed (Fig. 1B<sub>2</sub>). There were some aggregation of the external granular cells (Fig. 1B<sub>1</sub>) and the cells of the IGL had pyknotic nuclei with some degenerative changes (Fig. 1B<sub>4</sub>) at this stage.

Following the age to the end of the 2nd postnatal week, the cells of the ML were extremely damaged and their cytoplasm exhibited severe degree of granular degeneration (Fig. 2B<sub>2</sub>–B<sub>4</sub>) with numerous clear vacuoles denoting fatty degeneration (Fig. 2B<sub>4</sub>). A reduction in the size IGL with oedema (Fig. 2B<sub>5</sub>) was noticed. Also, there was some aggregation of cells of the EGL (Fig. 2B<sub>3</sub>). Fig. 2B<sub>4</sub> and B<sub>5</sub> showed many cells of the PL separated from its surrounding by clear area indicating infiltration of the oedematous fluid with pyknotic nuclei during this age. With the age progress to the end of the 3rd postnatal week, perivascular oedema between PL and IGL were noticed (Fig. 3B<sub>1</sub> and B<sub>2</sub>). Moreover, hyperplastic proliferation in the external granular cells was observed (Fig. 3B<sub>3</sub>). Vacuoles increment and pyknotic nuclei were recorded in most of the ML cells (Fig. 3B<sub>4</sub>) with reduction in the number of the PC (Fig. 3B<sub>3</sub>). Furthermore, the IGL exhibited severe degrees of granular and necrotic degeneration (Fig. 3B<sub>5</sub>) was noticed (Fig. 3B<sub>4</sub> and B<sub>5</sub>).

**3.2.1.3. In hyperthyroid group.** At the end of the 1st postnatal week, there was thickening in EGL (Fig. 1C<sub>2</sub> and C<sub>3</sub>) and some degeneration (Fig. 1C<sub>2</sub>). A reduction in the size of the ML was observed in Fig. 1C<sub>2</sub>–C<sub>4</sub>. In addition, the PCs displayed variable degrees of cell loss (Fig. 1C<sub>1</sub> and C<sub>4</sub>). Also, IGL were distended and fragmented with the presence of hyperemia (Fig. 1C<sub>1</sub> and C<sub>3</sub>) at this age. Sub-

sequently, as the age progressed to the end of the 2nd postnatal week, reduction in the size of the ML (Fig. 2C<sub>2</sub>), and distortion in the PCs were observed (Fig. 2C<sub>3</sub> and C<sub>4</sub>). The thickening of the EGL was observed in Fig. 2C<sub>2</sub> though the size of this layer was decreased in Fig. 2C<sub>1</sub>, C<sub>3</sub> and C<sub>4</sub>. More so, during this period, hyperemic blood vessels and degenerative symptoms in the IGL were observed (Fig. 2C<sub>3</sub>).

More so, with the age progress to the end of the 3rd postnatal week, the thickening of the EGL was observed (Fig. 3C<sub>4</sub>). Also, a reduction in number of PCs (Fig. 3C<sub>1</sub> and C<sub>2</sub>) with some vacuolation in the IGL (Fig. 3C<sub>3</sub>) were noticed. A hyperemic blood vessels and alterations in the PCs position (Fig. 3C<sub>2</sub>), oedematous areas (Fig. 3C<sub>4</sub>), degenerative symptoms (Fig. 3C<sub>1</sub>), and distended the cellular element of the IGL were observed at this postnatal week (Fig. 3C<sub>1</sub>–C<sub>4</sub>).

#### 3.2.2. Carbohydrate granules [periodic acid Schiff (PAS) reaction stain]: (Fig. 4)

The PAS reaction, in normal rat newborns, was step wisely increased during the first 3 postnatal weeks (Fig. 4A<sub>1</sub>–A<sub>3</sub>). Sections of the normal rat newborns frequently exhibited a more intense PAS reaction in comparison with the reaction in hypothyroid ones with the age progress (Fig. 4A<sub>1</sub>–A<sub>3</sub> and B<sub>1</sub>–B<sub>3</sub>). Moreover, in hyperthyroid rats, the carbohydrate granules (neutral mucopolysaccharides) were more precipitated at the end of the 1st postnatal week even though the precipitation of these granules were decreased in other tested periods as compared to the normal ones (Fig. 4A<sub>1</sub>–A<sub>3</sub> and C<sub>1</sub>–C<sub>3</sub>).

### 4. The cerebral cortex: (Figs. 5–8)

#### 4.1. General histology (haematoxylin and eosin stain): (Figs. 5–7)

##### 4.1.1. In normal group

The cerebral hemispheres form the major region of the brain and have smooth surfaces with no evident furrows. This cortex is protected by the meninges (outermost layer) throughout the experimental period (Figs. 5A<sub>1</sub>, 6A<sub>1</sub>, and 7A<sub>1</sub>). Furthermore, at different ages, the cells of this cortex appeared with the pyramidal perikaryon and their apical dendrites were perpendicular to the pial surface. These cells increased in size and shape from the end of the 1st to 3rd weeks old (Figs. 5A<sub>2</sub>, 6A<sub>2</sub>, and 7A<sub>2</sub>).

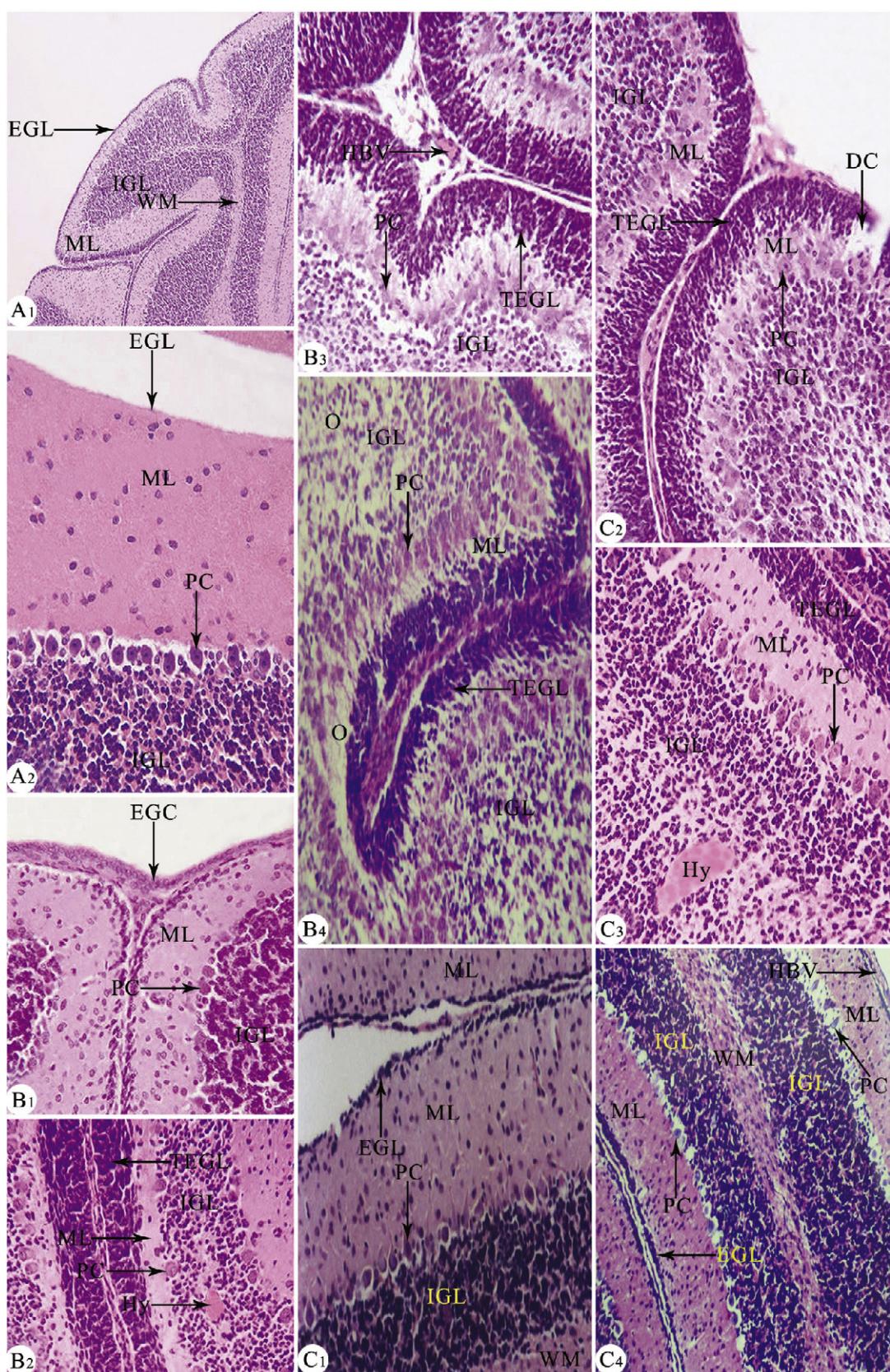
##### 4.1.2. In hypothyroid group

Regarding the newborns of this group, at the end of the 1st postnatal week, hyperplasia (Fig. 5B<sub>1</sub>) were observed in meninges. Moreover, Fig. 5B<sub>2</sub> and B<sub>3</sub> showed some degenerative changes and distortion in neurons of grey matter at this stage.

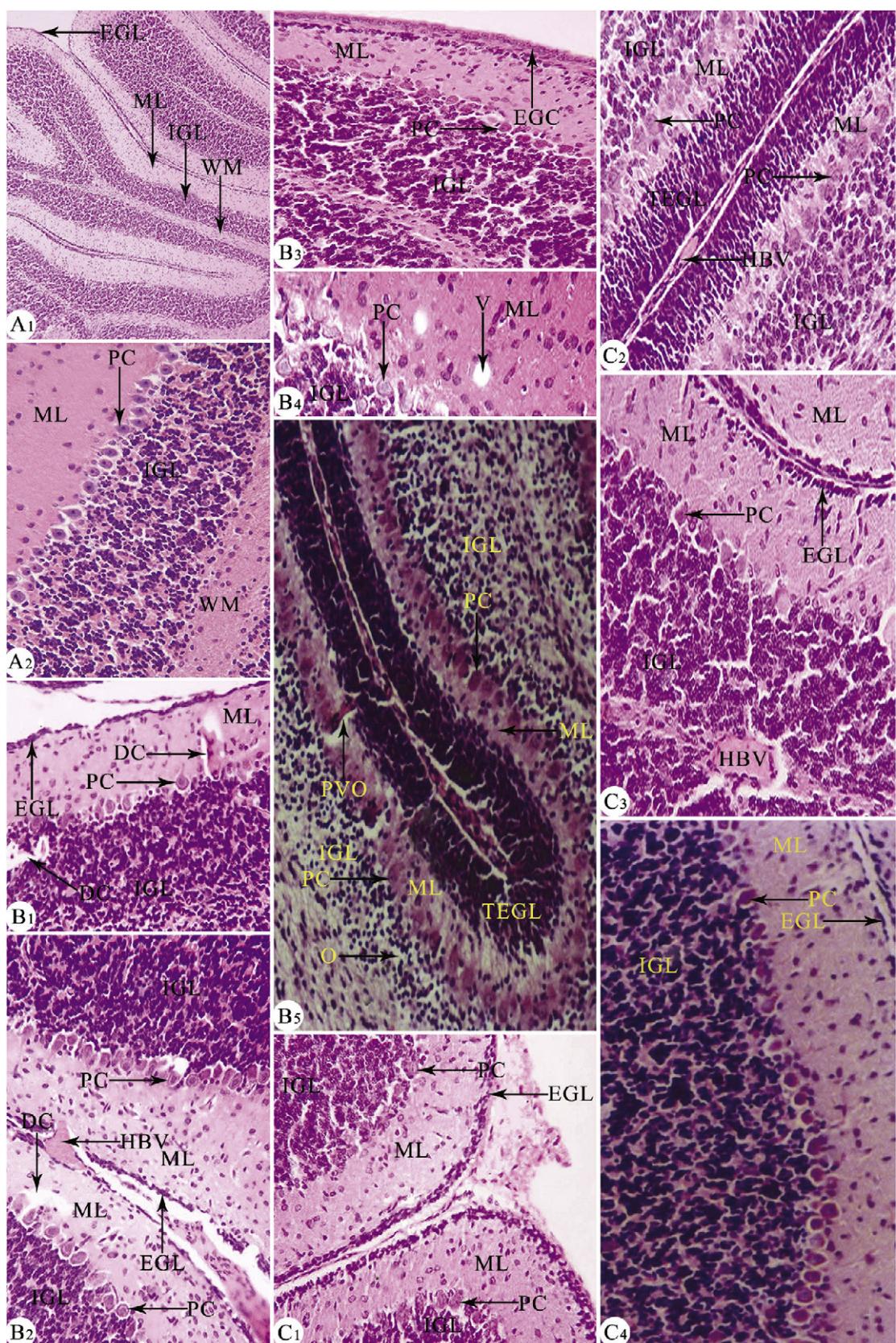
As the age progressed to the end of the 2nd postnatal week, some degeneration in the meninges (Fig. 6B<sub>1</sub>, B<sub>2</sub> and B<sub>4</sub>) was noticed. Furthermore, in grey matter, some degenerative changes (Fig. 6B<sub>3</sub> and B<sub>5</sub>), vacuoles (Fig. 6B<sub>4</sub> and B<sub>5</sub>), and some distortion in the pyramidal cells (PYCs) (Fig. 6B<sub>2</sub>–B<sub>5</sub>) were observed. As well, at the end of the 3rd postnatal week, the most severe degenerative effect and different patterns of damage were also present with some necrotic areas (Fig. 7B<sub>3</sub> and B<sub>5</sub>), vacuolation (Fig. 7B<sub>2</sub>, B<sub>4</sub> and B<sub>5</sub>) and inflammatory oedema in grey matter (Fig. 7B<sub>5</sub>) and the pyknotic-condensed nuclei were dispersed in the superficial layers whereas the shrinkage of the neurons and glial cells was distributed and apparently had suffered irreparable damage in the underlying layers (Fig. 7B<sub>3</sub>). Hyperemia (Fig. 7B<sub>1</sub> and B<sub>3</sub>) and thickening in meninges were noticed (Fig. 7B<sub>3</sub>). In general, some deformations in the meninges and PYCs (Fig. 7B<sub>2</sub>) were recorded at this postnatal week.

##### 4.1.3. In hyperthyroid group

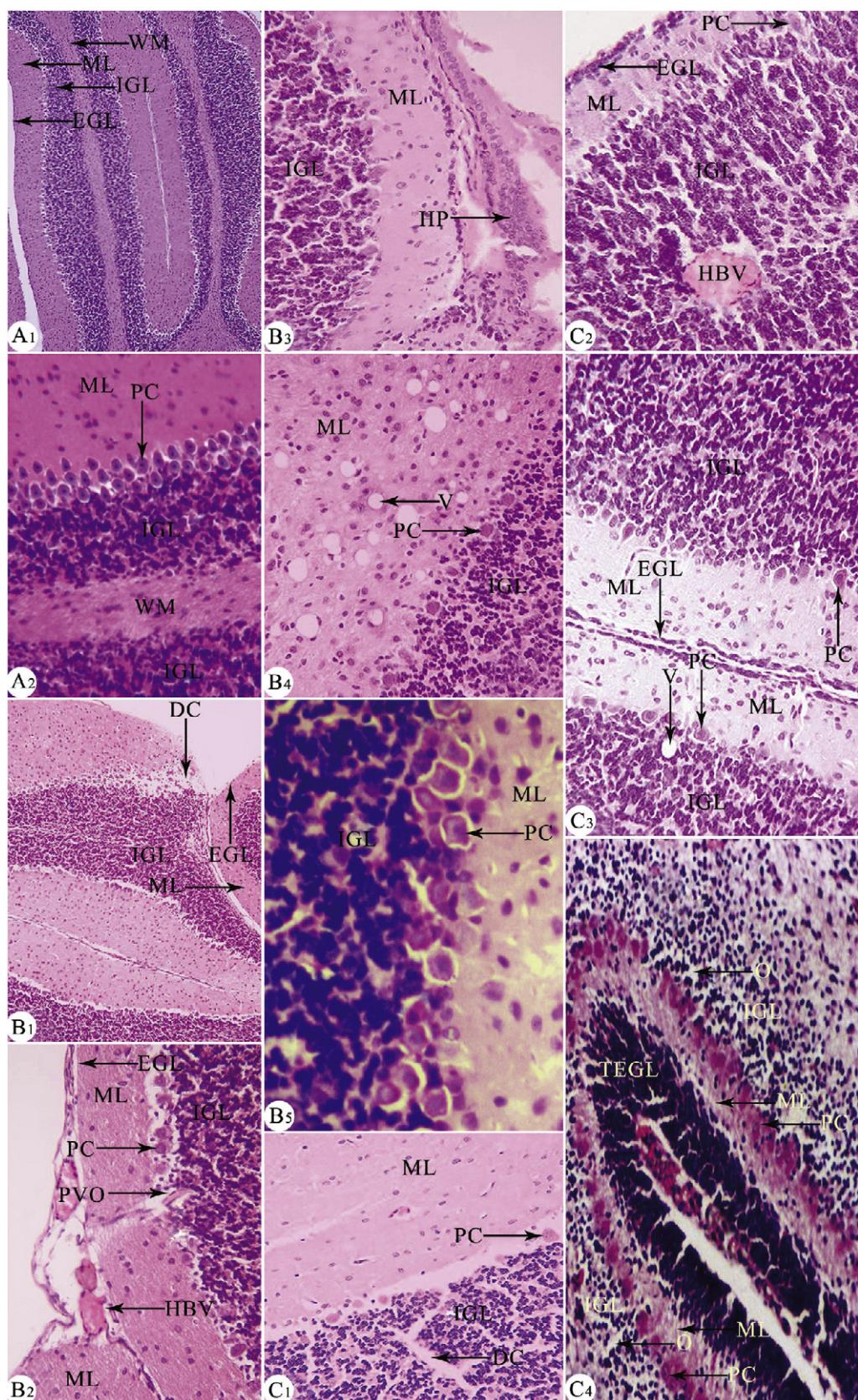
At the end of the 1st postnatal week, some degeneration were noticed in meninges (Fig. 5C<sub>1</sub>) and grey matter (Fig. 5C<sub>2</sub> and C<sub>3</sub>)



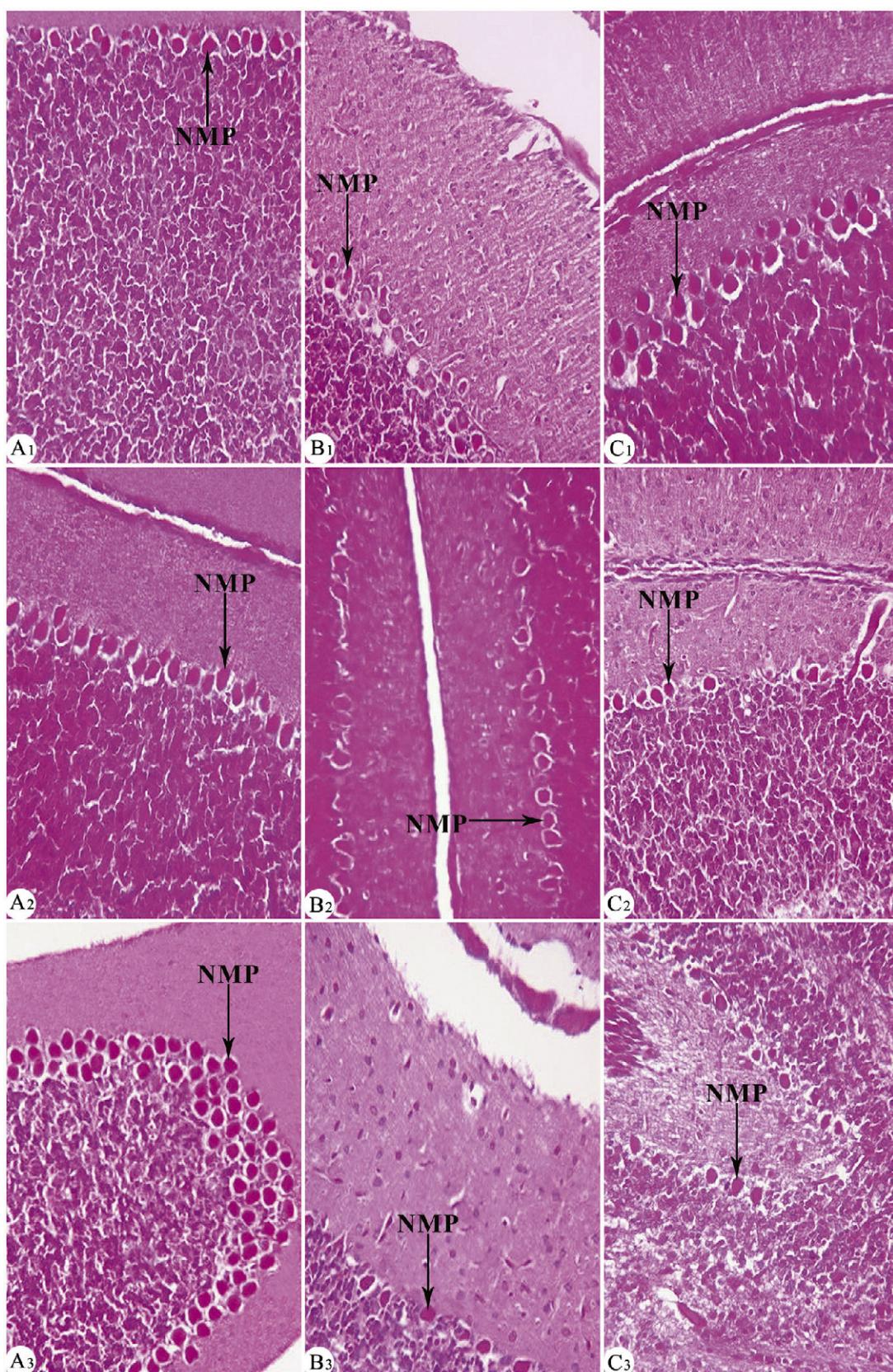
**Fig. 1.** Sagittal sections in the cerebellar cortex at the end of the 1st postnatal week in normal (A<sub>1</sub>,  $\times 100$  and A<sub>2</sub>,  $\times 400$ ), hypothyroid (B<sub>1</sub>–B<sub>3</sub>,  $\times 400$  and B<sub>4</sub>,  $\times 500$ ) and hyperthyroid rat newborns (C<sub>1</sub>–C<sub>3</sub>,  $\times 400$  and C<sub>4</sub>,  $\times 500$ ). (H&E stain).



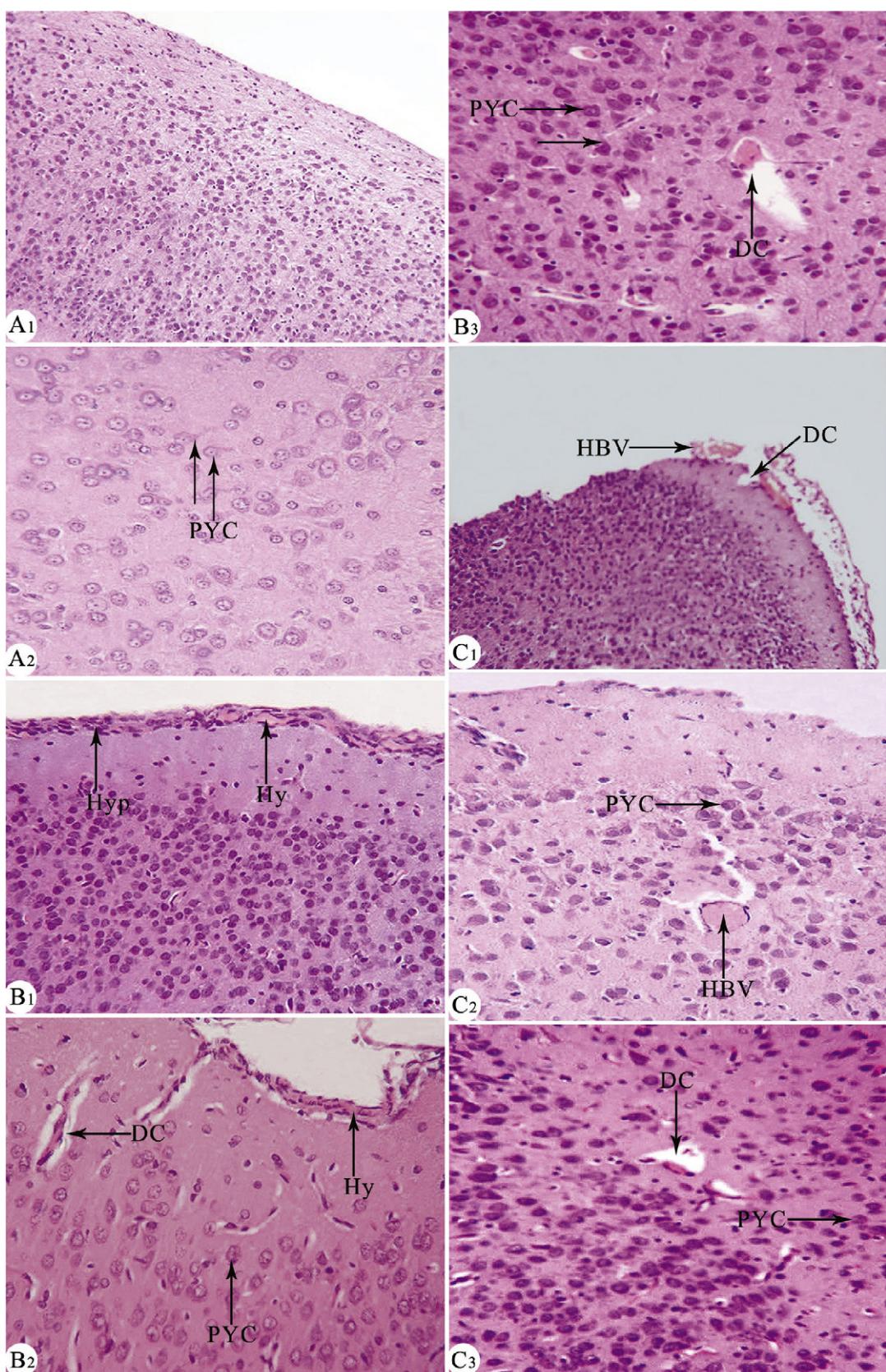
**Fig. 2.** Sagittal sections in the cerebellar cortex at the end of the 2nd postnatal week in normal (A<sub>1</sub>,  $\times 100$  and A<sub>2</sub>,  $\times 400$ ), hypothyroid (B<sub>1</sub>–B<sub>4</sub>,  $\times 400$  and B<sub>5</sub>,  $\times 500$ ) and hyperthyroid rat newborns (C<sub>1</sub>–C<sub>3</sub>,  $\times 400$  and C<sub>4</sub>,  $\times 500$ ). (H&E stain).



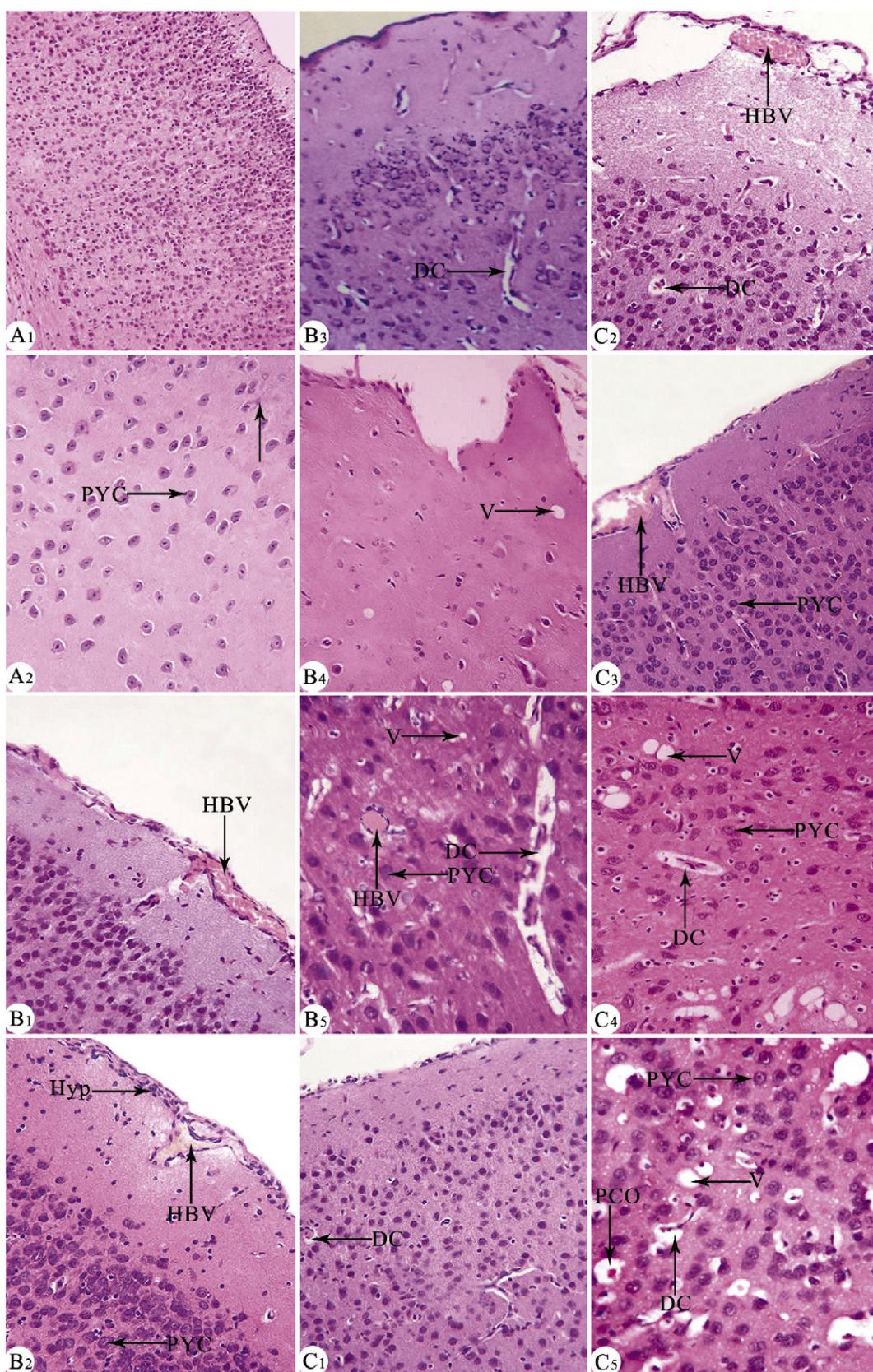
**Fig. 3.** Sagittal sections in the cerebellar cortex at the end of the 3rd postnatal week in normal (A<sub>1</sub>,  $\times 100$  and A<sub>2</sub>,  $\times 400$ ), hypothyroid (B<sub>1</sub>–B<sub>4</sub>,  $\times 400$  and B<sub>5</sub>,  $\times 500$ ) and hyperthyroid rat newborns (C<sub>1</sub>–C<sub>3</sub>,  $\times 400$  and C<sub>4</sub>,  $\times 500$ ). (H&E stain).



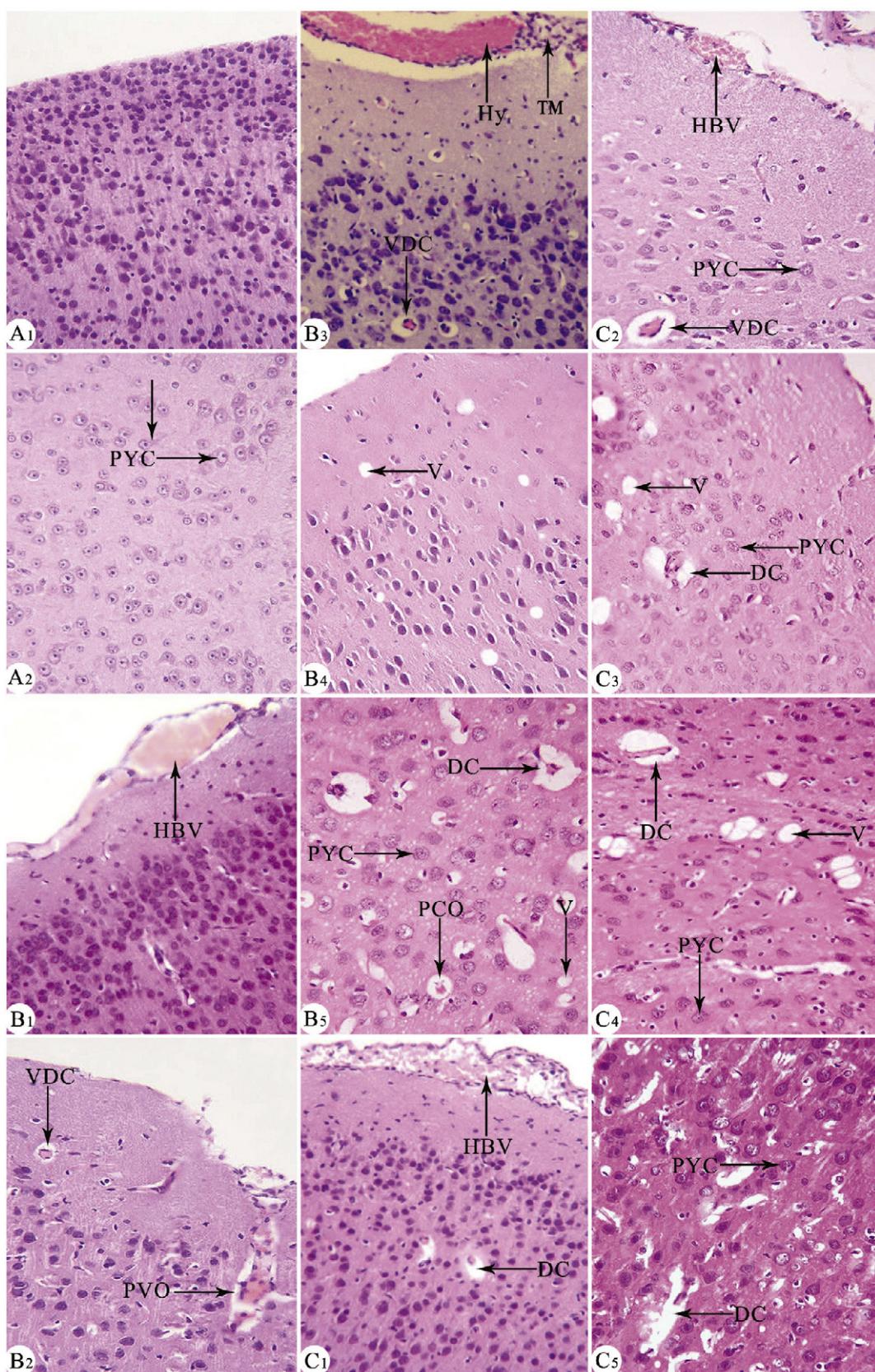
**Fig. 4.** Transverse sections in the cerebellar cortex of rat newborns showing the precipitations of neutral mucopolysaccharides (NMP). (PAS-Stain,  $\times 400$ ).  
 - At the end of the 1st postnatal week in normal (A<sub>1</sub>), hypothyroid (B<sub>1</sub>) and hyperthyroid newborns (C<sub>1</sub>).  
 - At the end of the 2nd postnatal week in normal (A<sub>2</sub>), hypothyroid (B<sub>2</sub>) and hyperthyroid newborns (C<sub>2</sub>).  
 - At the end of the 3rd postnatal week in normal (A<sub>3</sub>), hypothyroid (B<sub>3</sub>) and hyperthyroid newborns (C<sub>3</sub>).



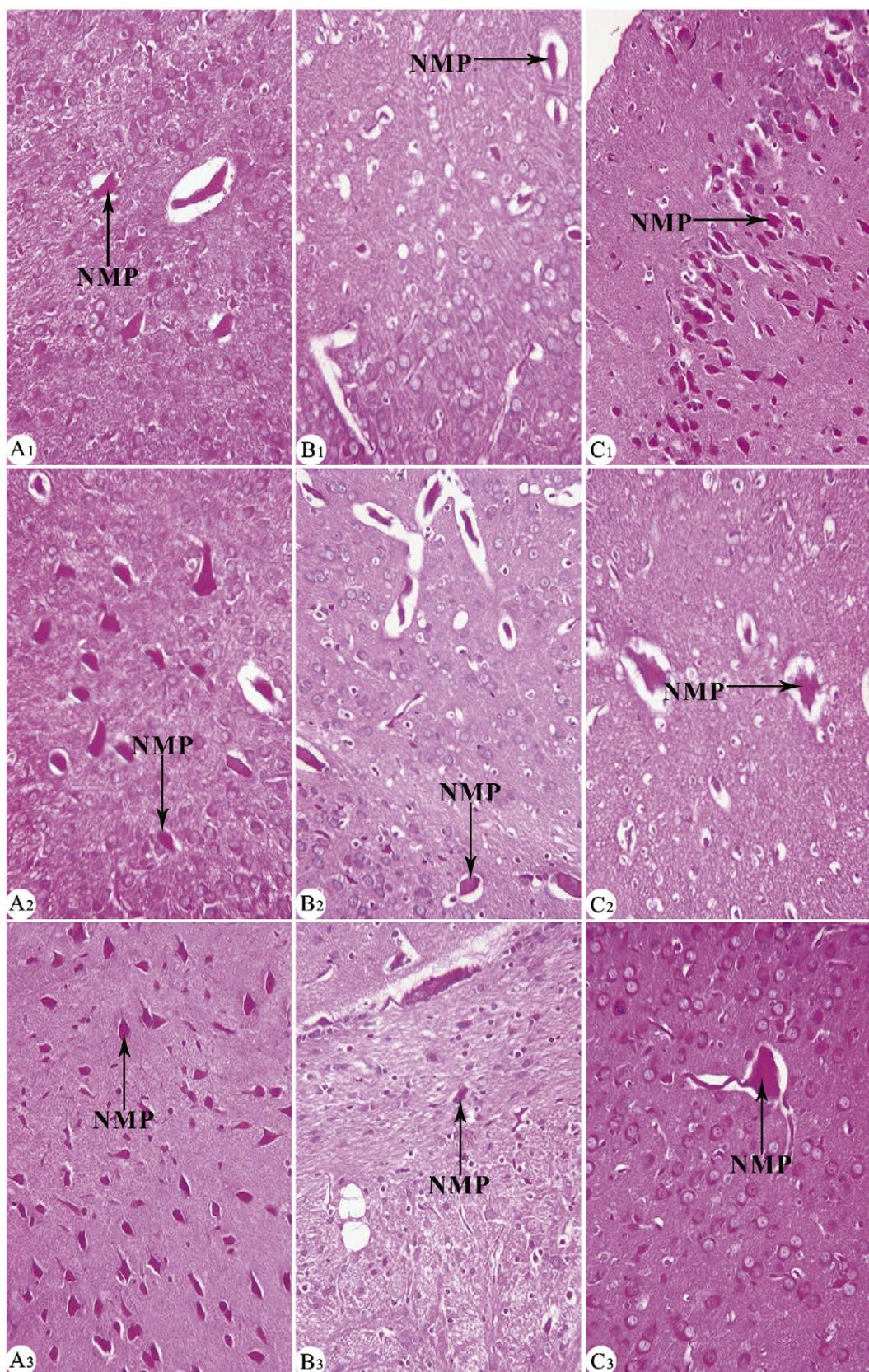
**Fig. 5.** Sagittal sections in the cerebral cortex at the end of the 1st postnatal week in normal (A<sub>1</sub>,  $\times 200$  and A<sub>2</sub>,  $\times 500$ ), hypothyroid (B<sub>1</sub>,  $\times 200$ ; B<sub>2</sub>,  $\times 400$  and B<sub>3</sub>,  $\times 500$ ) and hyperthyroid rat newborns (C<sub>1</sub>,  $\times 200$ ; C<sub>2</sub>,  $\times 400$  and C<sub>3</sub>,  $\times 500$ ). The arrows refer to the apical dendrites (H&E stain).



**Fig. 6.** Sagittal sections in the cerebral cortex at the end of the 2nd postnatal week in normal (A<sub>1</sub>,  $\times 200$  and A<sub>2</sub>,  $\times 500$ ), hypothyroid (B<sub>1</sub>,  $\times 200$ ; B<sub>2</sub>–B<sub>4</sub>,  $\times 400$  and B<sub>5</sub>,  $\times 500$ ) (H&E stain).



**Fig. 7.** Sagittal sections in the cerebral cortex at the end of the 3rd postnatal week in normal (A<sub>1</sub>,  $\times 200$  and A<sub>2</sub>,  $\times 500$ ), hypothyroid (B<sub>1</sub>,  $\times 200$ ; B<sub>2</sub>–B<sub>4</sub>,  $\times 400$  and B<sub>5</sub>,  $\times 500$ ) and hyperthyroid rat newborns (C<sub>1</sub>,  $\times 200$ ; C<sub>2</sub> and C<sub>3</sub>,  $\times 400$  and C<sub>4</sub> and C<sub>5</sub>,  $\times 500$ ). The arrows refer to the apical dendrites (H&E stain).



**Fig. 8.** Transverse sections in the cerebral cortex of rat newborns showing the precipitations of neutral mucopolysaccharides (NMP). (PAS-Stain,  $\times 400$ ).

- At the end of the 1st postnatal week in normal (A<sub>1</sub>), hypothyroid (B<sub>1</sub>) and hyperthyroid newborns (C<sub>1</sub>).
- At the end of the 2nd postnatal week in normal (A<sub>2</sub>), hypothyroid (B<sub>2</sub>) and hyperthyroid newborns (C<sub>2</sub>).
- At the end of the 3rd postnatal week in normal (A<sub>3</sub>), hypothyroid (B<sub>3</sub>) and hyperthyroid newborns (C<sub>3</sub>).

**Table 4**

Effect of thyroid status on body length (cm) of rat newborns.

Periods	Parameter Status	Body Length
1 Week	Control	5.933 ± 0.055 <sup>d</sup>
	Hypothyroid	4.801 ± 0.096 <sup>e</sup>
	Hyperthyroid	6.766 ± 0.151 <sup>c</sup>
2 Week	Control	6.633 ± 0.117 <sup>c</sup>
	Hypothyroid	5.765 ± 0.091 <sup>d</sup>
	Hyperthyroid	8.400 ± 0.203 <sup>b</sup>
3 Week	Control	8.246 ± 0.117 <sup>b</sup>
	Hypothyroid	6.832 ± 0.055 <sup>c</sup>
	Hyperthyroid	9.966 ± 0.091 <sup>a</sup>
LSD at 5% level		0.339
LSD at 1% level		0.457
F-probability		P < 0.001

- Data are expressed as mean ± SE. Number of animals in each group is six.

- Values which share the same superscript symbols are not significantly different.

- F-probability expresses the effect between groups, where P < 0.001 is very highly significant.

while at the end of the 2nd postnatal week, pericellular oedema (Fig. 6C<sub>5</sub>), vacuoles (Fig. 6C<sub>4</sub> and C<sub>5</sub>) and hyperemic blood vessels (Fig. 6C<sub>2</sub> and C<sub>3</sub>) were observed with some degeneration in the PYCs (Fig. 6C<sub>2</sub>–C<sub>5</sub>). Additionally, at the end of the 3rd postnatal week, many severely damaged neurons were found (Fig. 7C<sub>3</sub>–C<sub>5</sub>) associated with vacuoles increment (Fig. 7C<sub>3</sub> and C<sub>4</sub>). Also, some degenerative signs in meninges and grey matter were found at this age (Fig. 7C<sub>1</sub>–C<sub>5</sub>).

#### 4.2. Carbohydrate granules [periodic acid Schiff (PAS) reaction stain]: (Fig. 8)

Sections of the cerebral cortex, at the end of the 1st postnatal week, gave diffuse positive PAS reaction in normal rat newborns (Fig. 8A<sub>1</sub>). On the other hand, at the end of the 2nd and 3rd postnatal weeks, the cerebral cortex had more precipitated granules of PAS reaction than the previous age in normal rat newborns (Fig. 8A<sub>1</sub>–A<sub>3</sub>). In hypothyroid newborns, at all ages employed, the carbohydrate granules were less accumulated and precipitated as compared to the normal ones (Fig. 8A<sub>1</sub>–A<sub>3</sub> and B<sub>1</sub>–B<sub>3</sub>). Even though, in hyperthyroid newborns, the precipitation of the carbohydrate granules (neutral mucopolysaccharides) was increased at the end of the 1st postnatal week, this precipitation was decreased in other examined ages as compared with that of the control group (Fig. 8A<sub>1</sub>–A<sub>3</sub> and C<sub>1</sub>–C<sub>3</sub>).

#### 5. General gross morphology and X-Ray skeletal examinations (Tables 4 and 5 and Fig. 9)

X-ray examination showed that the growth of the skeletal features in normal rat newborns, were increased with the age progress to the end of the 3rd postnatal week (Fig. 9A<sub>1</sub>–A<sub>3</sub>). Moreover, the growth of these features, generally, were more developed in hyperthyroid group (Fig. 9C<sub>1</sub>–C<sub>3</sub>) whereas the opposite occurs in hypothyroid group (Fig. 9B<sub>1</sub>–B<sub>3</sub>) in comparison with their respective normal control along the duration of the experiment. Also, the congenital anomaly as kyphosis was observed in hypothyroid group at the end of the 1st and 3rd postnatal weeks only (Fig. 9B<sub>1</sub> and B<sub>3</sub>). Data obtained from body length of control and both treated groups were shown in Table 1. This variable was gradually increased in normal rat newborns with the age progress from the end of the 1st to 3rd weeks. Compared to controls, the mean values of these variables, during the experimental period, evidenced a highly significant decrease (LSD; P < 0.01) in hypothyroid group and increase (LSD; P < 0.01) in hyperthyroid group. These alterations, in both treated groups, were pronounced at the end of the 3rd postnatal week.

**Table 5**

Two-way analysis of variance (ANOVA) for body length of rat newborns.

Source of Variation	D.F.	F-Calculations Body Length
(1.) Control. Hypothyroid		
- General effect	5	160.603 ***
- Hypothyroid	1	228.610 ***
- Age	2	282.526 ***
- Hypothyroid X Age	2	4.676 **
- Error	30	
(2.) Control. Hyperthyroid		
- General effect	5	128.265 ***
- Hyperthyroid	1	178.536 ***
- Age	2	223.543 ***
- Hyperthyroid X Age	2	7.853 ***
- Error	30	

Where \* is non-significant (P > 0.05), \*\* is significant (P < 0.05), \*\*\* is highly significant (P < 0.01) and \*\*\*\* is very highly significant (P < 0.001).

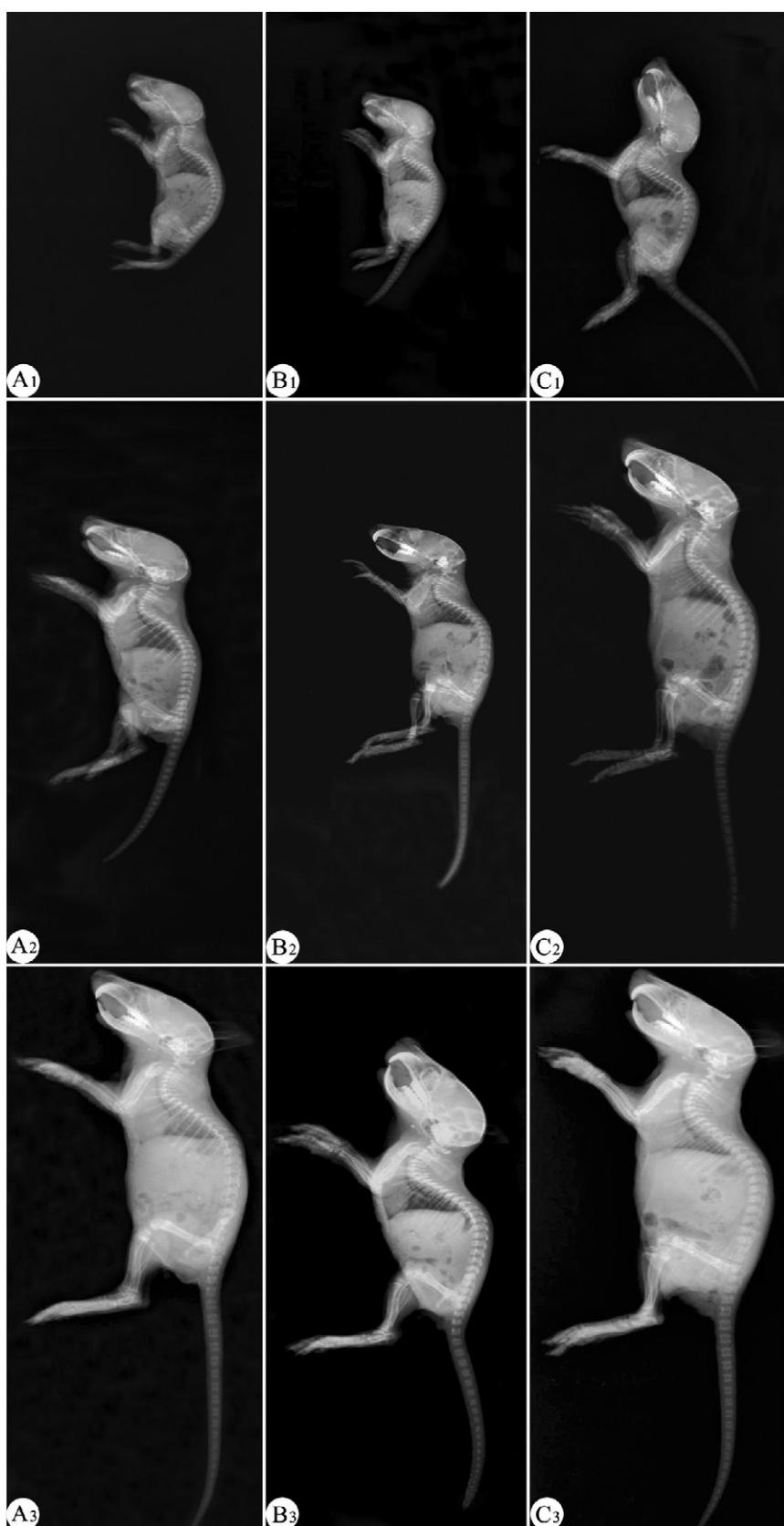
With regards one-way ANOVA of body length, it was demonstrated that the general effect between groups was very highly significant (P < 0.001) (Table 4). In addition, two-way analysis of variance revealed that the effect of age, hyperthyroidism and their interaction was very highly significant (Table 5). While the effect of age and hypothyroidism was very highly significant, their interaction was only significant (P < 0.05).

#### 6. Discussion

Because of thyroid hormones (THs) potentially influences brain development postnatally in the rat (Ahmed et al., 2008b), the current study highlights the effects of hypothyroid and hyperthyroid in different brain regions (cerebrum, cerebellum) of rat newborns at the end of the 1st, 2nd and 3rd postnatal weeks.

The present study revealed that administration of methimazole (MMI) in drinking water (0.02%, w/v) to adult female rats during pregnancy and weaning periods induced hypothyroidism in mothers and their newborns as indicated by decrease in serum total thyroxine (TT4), total triiodothyronine (TT3), free thyroxine (FT4) and free triiodothyronine (FT3) levels. This result coincides with several studies. Neonatal rats receiving the anti-thyroid drug as MMI that crosses the placenta and in their mother's milk, are rendered hypothyroid (MacNabb et al., 2000; Cristovao et al., 2002; Ramos et al., 2002; Mookadam et al., 2004; Hasebe et al., 2008). The mechanism by which MMI exerts hypothyroidism was explained by Awad (2002) and Ahmed et al. (2008b) who reported that MMI interferes with incorporation of iodine into tyrosyl residues of thyroglobulin (TG) and inhibits the coupling of iodothyrosyl residues to form iodothyronine, thus inhibiting the synthesis of THs. Similarly, propylthiouracil (PTU) produced a step-wise decrease in serum total T4, and a step-wise increase in serum TSH (Sharlin et al., 2009).

In our study, the administration of T4 to adult female rats in drinking water at 0.002%, w/v beside gastric intubation of 50–200 µg/kg body weight during pregnancy and weaning periods induced a marked hyperthyroidism in mothers and their newborns. This hyperthyroidism was ensured by elevated levels of serum TT3, TT4, FT3 and FT4 in these animals. Similarly to the MMI, THs from mothers may cross to the fetus through placenta or may pass in mother's milk to the neonates (Higuchi et al., 2005). Taking the previous information about the two experimental models together, it can be concluded that the mother's thyroid state during pregnancy and lactation periods affects the thyroid status of their newborns. This suggestion was supported by other publications (Varas et al., 2002; Ahmed et al., 2008b). Notably, several studies supposed (Awad, 2002; Ahmed et al., 2008b) that the thyroid function abnormalities in pregnancy can result in maternal hyper- or hypothyroidism. It can be suggested that the changes in the THs



**Fig. 9.** X-ray (6 ml ampere and 40 kvolt/0.5 second) examinations showing the general skeletal features of rat newborns.

- At the end of the 1st postnatal week in normal (A<sub>1</sub>), hypothyroid (B<sub>1</sub>) and hyperthyroid newborns (C<sub>1</sub>).
- At the end of the 2nd postnatal week in normal (A<sub>2</sub>), hypothyroid (B<sub>2</sub>) and hyperthyroid newborns (C<sub>2</sub>).
- At the end of the 3rd postnatal week in normal (A<sub>3</sub>), hypothyroid (B<sub>3</sub>) and hyperthyroid newborns (C<sub>3</sub>).

of adult female rats, in our experimental groups, may reflect the sensitivity of thyroid gland to the dose and duration.

In view of thyroid function of normal rat newborns, the current study revealed gradual increases of serum FT4, FT3 and thyrotropin (TSH) levels at the end of the 1st, 2nd and 3rd postnatal weeks. Also, growth hormone (GH) was markedly elevated in an age-dependent manner. These results are concomitant with those of Obregón et al. (1984) and Morreale de Escobar et al. (1985) who stated that after the fetal thyroid starts secreting THs, the fetal T4 and T3 pool as well as the circulating T4 level increased steadily until the thyroid gland is completely developed. With regards the normal maternal THs, in the present study, their serum levels were lower during the pregnancy at gestation day 10 than those at day 10 post-partum. This state may reflect the higher transfer of THs from pregnant females to their fetuses during pregnancy and/or more efficiency of thyroid gland to secret THs after birth. The steady increase in serum FT4 and FT3 levels with the age progress, in the present study, may reflect the histological changes in the thyroid gland which showed a marked and gradual increase in the size and number of thyroid follicles. The gradual increase of TSH is necessary for the development and growth of thyroid gland during this sensitive period because it increases the size of the follicles and it increases the rate of synthesis, secretion and iodination of glycoprotein into colloid, the rate of breakdown of thyroglobulin (TG) and the liberation of THs into circulation (Ahmed et al., 2008b). Furthermore, it was reported that the maturation of the pituitary-thyroid axis is intrinsically controlled by gestational age rather than by serum thyroid hormone levels (Hashimoto et al., 1991). Alternatively, THs, through their nuclear receptor, play a crucial role in regulating differentiation, growth, and metabolism in higher organisms (DeVito et al., 1999; Ahmed et al., 2008b). These hormones are also a necessary component for the physiological growth of a young organism, stimulating the secretion of GH and insulin-like growth factor (Wasniewska et al., 2003). In addition, GH is a key factor controlling postnatal growth and development (Zhou et al., 2005; Wong et al., 2006). In turn, these observations imply that the THs may regulate the growth and development, in part, via their influence on GH.

In view of the present study, the normal, hypo- and hyperthyroid rat newborns showed that most of folds and fissures of the cerebellum were defined at the end of the 1st week after birth. A similar picture was obtained by Millen et al. (1994) for normal rat brain. Also, the cerebellum was subdivided into 3–5 sets of folds by groups of fissures during the experimental period. These fissures run perpendicular to the antero-posterior axis. The central set makes up the vermis while the others equally make up lateral hemispheres and paraflocculi in normal newborns. These results go parallel with those of Getty (1975), Dalia (1998, 2002) and Ahmed (2004) after examining the cerebellum in some mammals. In our study, the cerebellar cortex consists of four layers in normal and both treated rats; the superficial external granular layer (EGL), molecular layer (ML), Purkinje layer (PL) and deep internal granular layer (IGL). These results are concomitant with the results of Dalia (1998, 2002) and Ahmed (2004) in white rat newborns and are parallel with the results of Gardner et al. (1976) in human's fetus. In fact, THs are required for normal maturation of cerebellum (Koibuchi and Chin, 2000; Bernal, 2003; Horn and Heuer, 2010). Moreover, in normal neurological development, these hormones act in the following manner (Porterfield, 1994): (1) they increase the rate of neuronal proliferation in the cerebellum (Dussault and Ruel, 1987; Bhanja and Chainy, 2010) and (2) they act as the "time clock" to end neuronal proliferation and stimulate differentiation (Dussault and Ruel, 1987; Pasquini and Adamo, 1994; Zhang et al., 2010).

On the other hand, in the study herein, the cerebral hemispheres of normal rat newborns form the major region of the brain and have

smooth surfaces with no evident furrows. This coincides with Eaton (1960) and Ahmed (2004) who speculated that the cerebral hemispheres in mammals become the largest part of the brain. These hemispheres appeared convoluted in modern ungulates, carnivores and primates, thus increasing the cortex allowing rooms for vast numbers of neurons and connections (Ahmed, 2004). More so, Dalia (1998, 2002) and Ahmed (2004) emphasized that the maturity of the cerebral cortex increased gradually with the age progress in rat newborns. On the other hand, De Escobarde et al. (2007) recorded that the cerebral cortex of the fetus depends on maternal T4 for the production of the T3 for nuclear receptor-binding and biological effectiveness. In rat, maternal TH crosses the placenta and is postulated to regulate fetal brain development (Sampson et al., 2000). Taken together, Zoeller (2007) reported that the influence of the THs on the normal function of the mammalian CNS depends on the brain region and on the developmental stage. Heuer (2007) concluded that THs are essential for proper brain development and function in mice. Introducing the same mode of thought, the experimental research and clinical studies have partially clarified the correlation between the maturation of the nervous system and thyroid function during the early stages of development; both a deficit and excess of THs may lead to permanent anatomo-functional damage to the CNS (Mussa et al., 1990). By the way, adult-onset thyroid disorders in humans impair several important central nervous system functions (Sarkar, 2008).

There were deformation and developmental defects in the cerebellar and cerebral cortex of the present hypo- and hyperthyroid rat newborns with the age progress. This observation concur with that proposed by Ahmed et al. (2008b) who suggested that any deficiency or increase of THs (hypo- or hyperthyroidism) during the development may result in an irreversible impairment, morphological and cytoarchitecture abnormalities, disorganization, maldevelopment and physical retardation for CNS. Wong and Leung (2001) confirmed that hypo- or hyperthyroidism affects the maturation of the CNS and causes irreversible dysfunction of the brain if not corrected shortly after the birth of both rodents and human. Moreover, Smith et al. (2002) said that the alterations in structure, function and behavior as a consequence of thyroid dysfunction, have highlighted the importance of these hormones, especially in CNS development and in the maintenance of neuronal system function throughout life. Pérez-López (2007) reported that iodine deficiency is the most avoidable cause of cerebral lesions including different degrees of mental retardation and cerebral paralysis. In general, iodine deficiency and hypothyroxinemia have a negative effect on the brain development during fetal (Akinci et al., 2006; De Escobarde et al., 2007) and early postnatal life (Akinci et al., 2006).

On the other hand, excess THs during the early stages of development may also cause permanent damage to the CNS (Mussa et al., 1990). Collectively, Lazarus (1999) and Zoeller and Crofton (2005) observed that in human, minor changes in maternal T4 are related to fetal brain maturation and development. In light of the previous observations with the present experiment, it seems logical to conclude that both hypo- and hyperthyroidism caused some malformation in the cerebellar and cerebral cortex of rat newborns.

Worth noting in the current study is that the cells of the superficial external granular layer (EGL) of normal rat newborns were difficult to be seen clearly from the 1st to 3rd weeks of birth. This result is interpreted as consistent with Altman and Anderson (1971) who recorded that the retardation in the development of IGL and ML reflects the delay in the differentiation of the cells of the EGL in rat. The damage of the EGL during this period has been shown to have a deleterious effect on the morphogenesis of the cerebellar cortex (Shimada and Langman, 1970) and on the cerebellar function (Shofer et al., 1964). In normal rats, mitotic activity

in the EGL declines with age and the EGL disappears at about 24 day (Nicholson and Altman, 1972a,b,c).

On the other hand, in the present hypo- and hyperthyroid groups, the histological sections showed that while the thickness of the EGL (aggregation of some external granular cells) was increased with the age progress, the degeneration was also observed throughout the experimental period. Lewis et al. (1976) found that in hypothyroid rats, the EGL reduced at 35 days of age and granule cells remain in a proliferative phase longer than in controls, resulting in decreased cell differentiation. Maternal hypothyroxinemia in mice clearly results in an alteration of neuronal migration during neocortogenesis (Tekki-Kessaris et al., 2001). In fact, hypothyroidism delays migration of cerebellar granule cells from the external germinative layer to the IGL in rat (Manzano et al., 2007).

On the other hand, hyperthyroidism (Nicholson and Altman, 1972a,b,c) in rats caused early termination of cell proliferation in the EGL accompanied by early disappearance of this layer. Hyperthyroidism in rat (Lauder et al., 1974) can cause the premature decline and disappearance of the EGL and decrease cortical growth. In hyperthyroid rat, Lauder (1977) speculated that cell division is initially increased in the EGL but then is prematurely terminated. In the forebrain of rat newborns, levothyroxine (L-T4) (3 µg by subcutaneous injection daily from birth) has no effect on cell proliferation in the first 6 postnatal days while it causes decreased cell acquisition from 12 to 21 days so that cell number becomes significantly reduced (Pate et al., 1979). In general, Rousseaux and Blokley (1991) concluded that cell populations with a high proliferative rate or those beginning to differentiate are the most susceptible to cytotoxicity. This finding may reflect the thickening in the external granular cells of cerebellar cortex and in the meninges of cerebral cortex in both treated groups of our work.

In view of the recent studies, the current results bring up an important findings, the development of the investigated brain regions in rat newborns are extremely sensitive to maternal hypo- and hyperthyroidism during the pregnancy period and the first 3 weeks of lactation period. Thus, both treatments may lead, in turn, to some harmful effect on the histological structures and neurons formation (some reduction in the cellular processes that grow from the neurons in different layers of the cerebellar and cerebral cortex). This drastic effect may be responsible for the neurons loss their vital functions (protein synthesis or metabolic rate). In conclusion, the present results suggest that, during normal development, THs have selective effects on histogenesis and the cytoarchitectural organization of the cerebellar and cerebral cortex. Any situation resulting in a decreased or increased the availability of THs to the fetal brain may potentially adverse for neurodevelopment.

The current investigation showed that the accumulation of the carbohydrates granules, in cerebral and cerebellar cortex of normal rat newborns, was found to be proportionally related to age progress from the 1st to 3rd postnatal weeks. This observation agrees with the results of Ahmed (2004) in cerebral and cerebellar cortex of rat newborns during the first four weeks after birth. In the early developmental stages, the neurons which are the most active metabolically might be expected to exhibit pigmentation at an earlier age than neurons which were less active (Ahmed, 2004). By year 2008, Ahmed et al. (2008a) reported that the maternal THs regulate the growth and metabolism of their newborns. As well, Tata (2006) postulated that in adult mammals and most vertebrates, THs control the basal metabolic rate and energy metabolism. Generally, Moreno et al. (2008) reported that the processes and pathways mediating the intermediary metabolism of carbohydrates, lipids, and proteins are all affected by THs in almost all tissues.

Furthermore, the carbohydrate granules of cerebral and cerebellar cortex were less precipitated in the present hypo- and hyperthyroid newborns during the first 3 postnatal weeks, except at the end of the 1st week in both regions of hyperthyroid group

only in relation to normal ones. Several attempts are recorded that the neonatal hypothyroidism results in diminished metabolic process (Arnold et al., 2003; Ahmed et al., 2008b). Furthermore, Hendrich et al. (1997) suggested that in human mothers, maternal hypothyroidism induces maternal metabolic dysfunction. On the other hand, hyperthyroidism is a hypermetabolic state (Mayer et al., 2004). The latter observation can be discussed according to the conclusion of Abdeen (1979) who speculated that low doses of T4 increase the rate of glycogen synthesis in rat. Alternatively, in patients, deficiency or excess of THs is associated with CNS disturbances (Ozata et al., 1996). It can be inferred from the above mentioned results that both thyroid states, in general, may alter the amount of nutrients reaching the cortex through its effects on the blood vessels; this may reduce the activity of the neurons via the impairment of the carbohydrates genesis and reflex some delaying in the growth of the cellular processes of nerve cells.

X-Ray examination, in our results, depicted that the skeletal growth and their differentiation were increased with the age progress in normal rat newborns. Also, the current investigation also showed that the body length of normal newborns was increased with the age progress. These observations agree with Komárková et al. (1967) and Awad (2002) who reported that bone turnover is increased during lactation in rats. Marie et al. (1986) recorded that both bone formation and osteoclast-mediated resorption increased during pregnancy in rats. In fetal rats, there is a progressive rise in total and ionized calcium over the last week of gestation (Thomas et al., 1981). THs affect the development of the skeletal system through effects on the initiation and fusion of ossification centers as well as on bone elongation (Legrand, 1986). Even more interesting is the elegant study discussed by Shellabarger (1964) who declared that the normal growth and development appear to depend upon a direct action of the THs and, in part, upon the action of other hormones, particularly GH acting in concert with the THs. GH appears to be primarily responsible, through IGF actions on cell proliferation, for cartilage growth in the epiphyseal plate of long bones (Awad, 2002).

In light of the current work, the skeletal bones, in all employed ages, were more developed in hyperthyroid group and the opposite occurs in hypothyroid group in relation to control group. In addition, at the end of the 1st and 3rd postnatal weeks, kyphosis was noted in hypothyroid group as congenital anomalies. Concurrently, the body length, in our study, was decreased in hypothyroid newborns and increased in hyperthyroid group during the experimental duration as compared to the data in the age-matched normal controls. Similar explanations are reported by several studies. Hypothyroidism, during the development, has an adverse effect on bone growth and results in a growth retardation in rats (Awad, 2002), mice (Wassef and Hassan, 1993) and human (Kvistad et al., 2004). Concomitantly, hypothyroidism can retard markedly the body growth in rats (Awad, 2002) and human (Kumar and Chaudhuri, 1990). It has been previously documented that the skeletal growth were increased in hyperthyroidism (Dziewiatkowski, 1951). Furthermore, untreated childhood thyrotoxicosis causes accelerated growth and advanced bone age with premature closure of the growth plate and short stature (Segni and Gorman, 2001). Hyperthyroidism also results in increasing the osteoclastic response to TH (Jowsey and Gorddan, 1971). Also, Kosińska et al. (2005) concluded that treatment with substitutive or suppressive doses of thyroxine stimulates osteogenic and osteoclastic processes in pre- and postmenopausal women.

With respect to these results, it must be considered that the thyroid dysfunction may be responsible for the alterations of general skeletal features and body length during the postnatal development; this may be due to the injurious effects of MMI and T4 on

the biological reactions. In this connection, several authors presumed that the disturbance through loss or predomination of one or more of the participating hormones leads to remarkable histological and chemical changes in cartilage and bone (Awad, 2002). Hormonal disturbance also leads to noticeable differences in the expansion of the epiphyseal cartilage centers and in the relation between these centers and the spongiosal zone (Greenberg et al., 1974). In summary, most of thyroid function disorders may result in reduced bone density and/or increased fracture rate that should be taken into consideration at clinical evaluation (Lakatos, 2003). Collectively, hypo- or hyperfunctioning of the thyroid gland can affect all organ systems, growth, development and long-term functioning (Awad, 2002; Ahmed et al., 2008b; Zhang et al., 2010).

The experimental work herein will require additional evidence at a molecular level either demonstrating a direct action of the thyroid hormones on the fetal brain or additional evidence supporting the suggestion that the observed effects of maternal hypo- or hyperthyroidism on fetal development are explained by impaired gestation. Thus, whether the adverse effects of maternal hypo- or hyperthyroidism on fetal development are mediated directly by loss of the maternal hormones contribution to the fetus, indirectly by metabolic impairment of gestation, or both. In addition, future attention should be focused on identifying a non-genomic approach because of there is scant evidence and these actions of thyroid hormones (THs) differ across the developmental time and brain region.

## References

- Abdeen, A.M., 1979. Histochemical and morphometric studies on the effect of thyroxine, parathyroid hormone and calcitonin on intracartilaginous ossification in the rat. M.Sc. Thesis. Fac. of Sci., Mansura Univ., Egypt.
- Abdel-Moneim, A.A., 2005. Effects of repaglinide administration on some biochemical and oxidative stress parameters of diabetic and hyperthyroid albino rats. *J. Egypt. Ger. Soc. Zool. Comp. Physiol.* 46 (A), 1–29.
- Ahmed, R.G., 2004. Effect of heat Stress on the development of the nervous system in albino rats. M.Sc. Thesis. Fac. of Sci., Beni-Suef Branch, Cairo Univ., Egypt.
- Ahmed, O.M., 2006. Evaluation of the antihyperglycemic, antihyperlipidemic and myocardial enhancing properties of pioglitazone in diabetic and hyperthyroid rats. *J. Egypt. Ger. Soc. Zool. Comp. Physiol.* 51 (A), 253–278.
- Ahmed, M., Sarwar, M., Ahmed, I., Qureshi, G.A., Makhdoom, A., Parvez, S.H., 2007. Effect of carbimazole induced hypothyroidism and thyroxine replacement on the growth of the long bones in albino rats of different age groups. *Neuroendocrinol. Lett.* 28 (4), 484–488.
- Ahmed, O.M., El-Gareib, A.W., El-bakry, A.M., Abd El-Tawab, S.M., Ahmed, R.G., 2008b. Thyroid hormones states and brain development interactions. *Int. J. Dev. Neurosci.* 26 (2), 147–209 (Review).
- Akinci, A., Sarac, K., Güngör, S., Mungan, I., Aydin, O., 2006. Brain MR spectroscopy findings in neonates with hypothyroidism born to mothers living in iodine-deficient areas. *AJNR Am. J. Neuroradiol.* 27 (10), 2083–2087.
- Altman, J., Anderson, W.J., 1971. Irradiation of the cerebellum in infant rats with low level X-ray: Histological and cytological effects during infancy and adulthood. *J. Exp. Neurol.* 30, 492–509.
- Arnold, A.M., Anderson, G.W., McIver, B., Eberhardt, N.L., 2003. A novel dynamin III isoforms is up-regulated in the central nervous system in hypothyroidism. *Int. J. Dev. Neurosci.* 21 (5), 267–275.
- Awad, M.F.I., 2002. Increased risk of fetal anomalies following maternally induced hypothyroidism in female albino rats. Ph.D. Thesis. Fac. of Sci., Cairo Univ., Egypt.
- Bancroft, J.D., Stevens, A., 1982. Theory and Practical Techniques, second ed. Churchill-Livingstone Edinburg, London, Melbourne, New York.
- Bernal, J., 2003. Thyroid hormone and the brain: Target cells, role of receptors, and timing of action. *Hot Thyroidology* ([www.hothyroidology.com](http://www.hothyroidology.com)), October, No. 2, 2003.
- Bhanja, S., Chainy, G.B., 2010. PTU-induced hypothyroidism modulates antioxidant defence status in the developing cerebellum. *Int. J. Dev. Neurosci.* 28 (3), 251–262.
- Broedel, O., Eravci, M., Fuxius, S., Smolarz, T., Jeitner, A., Grau, H., Stoltenburg-Didinger, G., Plueckhan, H., Meinhold, H., Baumgartner, A., 2003. Effects of hyper- and hypothyroidism on thyroid hormone concentrations in regions of the rat brain. *Am. J. Physiol. Endocrinol. Metab.* 285, E470–E480.
- Bruno, A.N., Ricchenevsky, F.K., Pochmann, D., Bonan, C.D., Battastini, A.M., Barreto-Chaves, M.L., Sarkis, J.J., 2005. Hypothyroidism changes adenine nucleotide hydrolysis in synaptosomes from hippocampus and cerebral cortex of rats in different phases of development. *Int. J. Dev. Neurosci.* 23 (1), 37–44.
- Carageorgiou, H., Pantos, C., Zarros, A., Stolakis, V., Mourouzis, I., Cokkinos, D., Tsakiris, S., 2007. Changes in acetylcholinesterase, Na<sup>+</sup>,K<sup>+</sup>-ATPase, and Mg<sup>2+</sup>-ATPase activities in the frontal cortex and the hippocampus of hyper- and hypothyroid adult rats. *Metabolism* 56 (8), 1104–1110.
- Chiesa, A., Gruneiro, d.P.L., Keselman, A., Heinrich, J.J., Bergada, C., 1994. Growth follow-up in 100 children with congenital hypothyroidism before and during treatment. *J. Pediatr. Endocrinol.* 7, 211–217.
- Cristovao, F.C., Bisi, H., Mendonca, B.B., Bianco, A.C., Bloise, W., 2002. Severe and mild neonatal hypothyroidism mediate opposite effects on Leydig cells of rats. *Thyroid* 12, 13–18.
- Dalia, M.S., 1998. Comparative neuroanatomical studies on the brain development in small mammals under the effect of high temperature. M.Sc. Thesis. Fac. of Sci., Mansura Univ., Egypt.
- Dalia, M.S., 2002. Comparative studies on the ontogeny of sensorimotor reflexes and locomotive activity in small mammals and their applications on infants. Ph.D. Thesis. Fac. of Sci., Mansura Univ., Egypt.
- De Escobarde, G.M., Obregón, M.J., del Rey, F.E., 2007. Iodine deficiency and brain development in the first half of pregnancy. *Public Health Nutr.* 10 (12A), 1554–1570.
- DeVito, M., Biegel, L., Brouwer, A., Brown, S., Brucker-Davis, F., Cheek, A.O., Christensen, R., Colborn, T., Cooke, P., Crissman, J., Crofton, K., Doerge, D., Gray, E., Hauser, P., Hurley, P., Kohn, M., Lazar, J., McMaster, S., McClain, M., McConnell, E., Meier, C., Miller, R., Tietge, J., Tyl, R., 1999. Screening methods for thyroid hormone disruptors. *Environ. Health Perspect.* 107 (5), 407–415. Review.
- Duffell, S.J., Soames, A.R., Gunby, S., 2000. Morphometric analysis of the developing rat brain. *Toxicol. Pathol.* 28 (1), 157–163.
- Dussault, J.H., Ruel, J., 1987. Thyroid hormone and brain development. *Annu. Rev. Physiol.* 49, 321–334.
- Dziewiakowski, D.D., 1951. Effect of thyroxine and thiouracil on S<sup>35</sup> deposition in articular cartilage. *J. Biol. Chem.* 189, 717–727.
- Eaton, T.H., 1960. Comparative Anatomy of the Vertebrates, second ed. Harper and Brothers, New York.
- Freitas, F.R.S., Capelo, L.P., O'Shea, P.J., Jorgetti, V., Moriscot, A.S., Scanlan, T.S., Williams, G.R., Zorn, T.M.T., Gouveia, C.H.A., 2005. The thyroid hormone receptor β-specific agonist GC-1 selectively affects the bone development of hypothyroid rats. *J. Bone Miner. Res.* 20 (2), 294–304.
- Gardner, D.L., Dodds, T.C., Royd, J.D., 1976. Human Histology, third ed. Churchill Livingstone, New York.
- Getty, R., 1975. The Anatomy of the Domestic Animals, fifth ed. W.B. Saunders Co., London.
- Gilbert, M.E., Sui, L., 2006. Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. *Brain Res. Arch.*, 10–22.
- Greenberg, A.H., Najjar, S., Blizard, R.M., 1974. Effect of thyroid hormone on growth, differentiation and development. In: Greer, M.A., Solomon, D.H. (Eds.), *Handbook of Physiology*, section 7: endocrinology, vol. III: thyroid. Am. Physiol. Soc., Washington D.C., pp. 377–389.
- Guerrero, A., Palmpona, R., Portero-Otín, M., Baja, G., López-Torres, M., 1999. Effect of thyroid status on lipid composition and peroxidation in the mouse liver. *Free Radic. Biol. Med.* 26 (1–2), 73–80.
- Hasebe, M., Matsumoto, I., Imagawa, T., Uehara, M., 2008. Effects of an anti-thyroid drug, methimazole, administration to rat dams on the cerebellar cortex development in their pups. *Int. J. Dev. Neurosci.* 26 (5), 409–414.
- Hashimoto, H., Sato, T., Horita, S., Kubo, M., Ohki, T., 1991. Maturation of the pituitary-thyroid axis during the perinatal period. *Endocrinol. Jpn.* 38 (2), 151–157.
- Hendrich, C.E., Ocasio-Torres, W., Berdecia-Rodriguez, J., Wiedmeier, V.T., Porterfield, S.P., 1997. Thyroid hormone regulation of brain amino acid utilisation and protein synthesis in fetuses and progenies of hypothyroid mothers – a review. In: Hendrich, C.E. (Ed.), *Recent Research Developments in Neuroendocrinology—Thyroid Hormone and Brain Maturation*. Research Signpost, India, pp. 87–102.
- Heuer, H., 2007. The importance of thyroid hormone transporters for brain development and function. *Best Pract. Res. Clin. Endocrinol. Metab.* 21 (2), 265–276.
- Higuchi, R., Miyawaki, M., Kumagai, T., Okutani, T., Shima, Y., Yoshiyama, M., Ban, H., Yoshikawa, N., 2005. Central hypothyroidism in infants who were born to mothers with thyrotoxicosis before 32 weeks gestation: 3 cases. *J. Pediatr.* 115 (5), e623–e625.
- Hoch, F.L., 1974. Metabolic effects of thyroid hormones. In: Greep and Astwood, *Handbook of physiology*, section 7: Endocrinology, vol. III: Thyroid. Am. Physiol. Soc., Washington D.C., pp. 391–412.
- Horn, S., Heuer, H., 2010. Thyroid hormone action during brain development: more questions than answers. *Mol. Cell Endocrinol.* 315 (1–2), 19–26 (Epub 2009 September 16).
- Jowsey, J., Gordean, G., 1971. Bone turnover and osteoporosis. In: *The Biochemistry and Physiology of Bone*, 2nd edition, by Bourne, G.H., vol. III, development and growth, Academic Press, New York/London, p. 204.
- Klein, A.H., Meltzer, S., Kenny, F.M., 1972. Improved prognosis in congenital hypothyroidism treated before age three months. *J. Pediatr.* 81, 912–915.
- Koibuchi, N., Chin, W.W., 2000. Thyroid hormone action and brain development. *Trends Endocrinol. Metab.* 11, 123–128.
- Komárková, A., Záhor, Z., Czabanová, V., 1967. The effect of lactation on the composition of long bones in rats. *J. Lab. Clin. Med.* 69, 102–109.
- Kosińska, A., Syrenicz, A., Syrenicz, M., Kosiński, B., Miazgowski, T., Garanty-Bogacka, B., 2005. The influence of treatment with substitutive or suppressive doses of thyroxine on biochemical bone turnover markers. *Ann. Acad. Med. Stetin.* 51 (2), 97–104.

- Kumar, R., Chaudhuri, B.N., 1990. Altered maternal thyroid function: fetal and neonatal myocardial metabolism. *Biol. Neonate* 57 (5), 300–312.
- Kvistad, P.H., Lovas, K., Boman, H., Myking, O.L., 2004. Retarded bone growth in thyroid hormone resistance. A clinical study of a large family with a novel thyroid hormone receptor mutation. *Eur. J. Endocrinol.* 150 (4), 425–430.
- Lakatos, P., 2003. Thyroid hormones: Beneficial or deleterious for bone? *Calcif. Tissue Int.* 73 (3), 205–209.
- Larsen, P.R., 1982. Thyroid-pituitary interaction; feedback regulation of thyrotropin secretion by thyroid hormones. *N. Engl. J. Med.* 306, 23–32.
- Lauder, J.M., 1977. Effects of thyroid state on development of rat cerebellar cortex. In: Grave, G.D. (Ed.), *Thyroid Hormone and Brain Development*. Raven Press, New York, pp. 235–254.
- Lauder, J.M., Altman, J., Krebs, H., 1974. Some mechanisms of cerebellar foliation: Effects of early hypo- and hyperthyroidism. *Brain Res.* 76 (1), 33–40.
- Lazarus, J.H., 1999. Thyroid hormone and intellectual development: a clinician's view. *Thyroid* 9 (7), 659–660.
- Lee, P.R., Brady, D., Koenig, J.I., 2003. Thyroid hormone regulation of N-Methyl-D-aspartic acid receptor subunit mRNA expression in adult brain. *Neuroendocrinology* 15 (1), 87–92.
- Legrand, J., 1967a. Analysis of the morphogenetic action of thyroid hormones on the cerebellum of young rats. *Arch Anat. Microsc. Morphol. Exp.* 56, 205–244.
- Legrand, J., 1967b. Variations, as a function of age, of the response of the cerebellum to the morphogenetic action of the thyroid in rats. *Arch. D Anat. Microsc. Morphol. Exp.* 56, 291–307.
- Legrand, J., 1986. Thyroid hormone effects on growth and development. In: Henneman, G. (Ed.), *Thyroid Hormone Metabolism*. Dekker, M., Inc., New York, pp. 503–534.
- Lewis, P.D., Patel, A.J., Johnson, A.L., Balazs, R., 1976. Effect of thyroid deficiency on cell acquisition in the postnatal rat brain: a quantitative histological study. *Brain Res.* 104, 49–62.
- MacNabb, C., O'Hare, E., Cleary, J., Georgopoulos, A.P., 2000. Varied duration of congenital hypothyroidism potentiates preservation in a response alteration discrimination task. *Neurosci. Res.* 36 (2), 121–127.
- Maes, M., Mommen, K., Hendrickx, D., Peeters, D., D'Hondt, P., Ranjan, R., De Meyer, F., Scharpé, S., 1997. Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. *Clin. Endocrinol. (Oxford)* 46 (5), 587–598.
- Mandel, S.J., Brent, G.A., Larsen, P.R., 1993. Levothyroxine therapy in patients with thyroid disease. *Ann. Intern. Med.* 119, 492–502. Also, available at [http://www.nacb.org/lmpg/thyroid\\_LMPG.Word.stm](http://www.nacb.org/lmpg/thyroid_LMPG.Word.stm) (accessed January 2005).
- Manzano, J., Bernal, J., Morte, B., 2007. Influence of thyroid hormones on maturation of rat cerebellar astrocytes. *Int. J. Dev. Neurosci.* 25 (3), 171–179.
- Marie, P.J., Cancela, L., Le Boulch, N., Miravet, L., 1986. Bone changes due to pregnancy and lactation: Influence of vitamin D status. *Am. J. Physiol.* 251, E400–E406.
- Mayer, L., Romic, Z., Skreb, F., Bacic-Vrca, V., Cepelak, I., Zanic-Grubisic, T., Kirin, M., 2004. Antioxidants in patients with hyperthyroidism. *Clin. Chem. Lab. Med.* 42 (2), 154–158.
- Millen, K.J., Wurst, W., Herrup, K., Joyner, A., 1994. Abnormal embryonic cerebellar development and patterning of postnatal foliation in two mouse engrailed-2 mutant. *Development* 120, 695–706.
- Mookadam, M., Leske, D.A., Fautsch, M.P., Lanier, W.L., Holmes, J.M., 2004. The anti-thyroid drug methimazole induces neo-vascularization in the neonatal rat analogous to ROP. *Invest. Ophthalmol. Vis. Sci.* 45, 4145–4150.
- Moreno, M., de Lange, P., Lombardi, A., Silvestri, E., Lanni, A., Goglia, F., 2008. Metabolic effects of thyroid hormone derivatives. *Thyroid* 18 (2), 239–253.
- Morreale de Escobar, G., Pastor, R., Obregón, M.J., Escobar del Rey, F., 1985. Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues before and after onset of fetal thyroid function. *Endocrinology* 117 (5), 1890–1901.
- Mussa, G.C., Zaffaroni, M., Mussa, F., 1990. Thyroid hormones and the development of the nervous system. *Minerva Pediatr.* 42 (9), 321–329.
- Nicholson, J.L., Altman, J., 1972a. The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. I: Cell proliferation and differentiation. *Brain Res.* 44, 13–23.
- Nicholson, J.L., Altman, J., 1972b. Synaptogenesis in the rat cerebellum: effects of early hypo- and hyperthyroidism. *Science* 176, 530–532.
- Nicholson, J.L., Altman, J., 1972c. The effects of early hypo- and hyperthyroidism on the development of the rat cerebellar cortex. II. Synaptogenesis in the molecular layer. *Brain Res.* 44, 25–36.
- Nunez, J., 1984. Effects of thyroid hormones during brain differentiation. *Mol. Cell. Endocrinol.* 37, 125–132.
- Obregón, M.J., Mallol, J., Pastor, R., Morreale de Escobar, G., Escobar del Rey, F., 1984. L-thyroxine and 3,5,3'-triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. *Endocrinology* 114, 305–308.
- O'Shea, P.J., Harvey, C.B., Suzuki, H., Kaneshige, M., Kaneshige, K., Cheng, S.-Y., Williams, G.R., 2003. A thyrotoxic skeletal phenotype of advanced bone formation in mice with resistance to thyroid hormone. *Mol. Endocrinol.* 17 (7), 1410–1424.
- Ornellas, D.S., Grozovsky, R., Goldenberg, R.C., Carvalho, D.P., Fong, P., Guggino, W.B., Morales, M., 2003. Thyroid hormone modulates ClC-2 chloride channel gene expression in rat renal proximal tubules. *Endocrinology* 178, 503–511.
- Ozata, M., Ozkardes, A., Dolu, H., Corakçioğlu, A., Yardim, M., Gundogan, M.A., 1996. Evaluation of central motor conduction in hypothyroid and hyperthyroid patients. *Endocrinol. Invest.* 19, 670–677.
- Pasquini, J.M., Adamo, A.M., 1994. Thyroid hormones and the central nervous system. *Dev. Neurosci.* 16 (1–2), 1–8.
- Patel, A.J., Lewis, P.D., Balázs, R., Bailey, P., Lai, M., 1979. Effects of thyroxine on postnatal cell acquisition in the rat brain. *Brain Res.* 172 (1), 57–72.
- Pérez-López, F.R., 2007. Iodine and thyroid hormones during pregnancy and postpartum. *Gynecol. Endocrinol.* 23 (7), 414–428.
- Porterfield, S.P., 1994. Vulnerability of the developing brain to thyroid abnormalities: Environmental insults to the thyroid system. *Environ. Health Perspect.* 102 (Suppl. 2), 125–130.
- Ramos, S., Goya, L., Martin, M.A., Escrivá, F., Pascual-Leone, A.M., 2002. Influence of hypothyroidism on circulating concentrations and liver expression of IGF-binding proteins mRNA from neonatal and adult rats. *Endocrinology* 172, 363–373.
- Reutens, A.T., 1995. Evaluation and application of a highly sensitive assay for serum growth hormone (GH) in the study of adult GH deficiency. *J. Clin. Endocrinol. Metab.* 80 (2), 480–485.
- Rousseaux, C.G., Blokley, P.M., 1991. The fetus. In: Haschek, W.M., Rousseaux, C.G. (Eds.), *Handbook of Toxicologic Pathology*. Academic Press Inc, pp. 938–981.
- Sampson, D., Pickard, M.R., Sinha, A.K., Evans, I.M., Leonard, A.J., Ekins, R.P., 2000. Maternal thyroid status regulates the expression of neuronal and astrocytic cytoskeletal proteins in the fetal brain. *Endocrinology* 141 (3), 439–445.
- Sarkar, P.K., 2008. L-triiodothyronine differentially and nongenomically regulates synaptosomal protein phosphorylation in adult rat brain cerebral cortex: role of calcium and calmodulin. *Life Sci.* 82 (17–18), 920–927.
- Sbaihi, M., Kacem, A., Aroua, S., Balache, S., Rousseau, K., Lopez, E., Meunier, F., Dufour, S., 2007. Thyroid hormone-induced demineralisation of the vertebral skeleton of the eel, *Anguilla anguilla*. *Gen. Comp. Endocrinol.* 151 (1), 98–107.
- Schwartz, H.L., Ross, M., Oppenheimer, J.H., 1997. Lack of effects of thyroid hormone on late fetal rat brain development. *Endocrinology* 138, 3119–3124.
- Segni, M., Gorman, C.A., 2001. The aftermath of childhood hyperthyroidism. *J. Pediatr. Endocrinol. Metab.* 14 (Suppl. 5), 1277–1278.
- Sharlin, D.S., Gilbert, M.E., Taylor, M., Ferguson, D., Zoeller, R.T., 2009. The nature of the compensatory response to low thyroid hormone in the developing brain. *J. Neuroendocrinol.*
- Shellabarger, C.J., 1964. The effect of thyroid hormones on growth and differentiation. In: Pitt-Rivers, R., Trotter, W.R. (Eds.), *The Thyroid Gland*, vol. 1. London.
- Shimada, M., Langman, J., 1970. Repair of the external granular layer after postnatal treatment with 5-fluorodeoxyuridine. *Am. J. Anat.* 129, 247–260.
- Shofer, R.J., Pappas, G.D., Purpura, D.P., 1964. Radiation induced changes in morphological and physiological properties of immature cerebellar cortex. In: Haley, T.J., Snider, R.S. (Eds.), *Response of the Nervous System to Ionizing Radiation*. Little, Brown, Boston, pp. 476–508.
- Smals, A.G.H., Ross, A.H., Kloppenborg, P.W.C., 1981. Dichotomy between serum free triiodothyronine and free thyroxine concentrations in familial thyroxine-binding globulin deficiency. *J. Clin. Endocrinol. Metab.* 53, 917–922.
- Smith, J.W., Evans, A.T., Costall, B., Smyth, J.W., 2002. Thyroid hormones, brain function and cognition: a brief review. *Neurosci. Behav. Rev.* 26 (1), 45–60.
- Süha, A., Çalikoğlu, M.D., 1999. Effects of thyroid hormones on central nervous system development. *Gazi Med. J.* 10, 3–10.
- Tata, J.R., 2006. Amphibian metamorphosis as a model for the developmental actions of thyroid hormone. *Mol. Cell. Endocrinol.* 246, 10–20.
- Tekki-Kessaris, N., Woodruff, R., Hall, A.C., Gaffield, W., Kimura, S., Stiles, C.D., Rowitch, D.H., Richardson, W.D., 2001. Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon. *Development* 128 (13), 2545–2554.
- Thakur, C., Saikia, T.C., Yadav, R.N., 1997. Total serum levels of triiodothyronine (T3), thyroxine (T4) and thyrotropin (TSH) in school going children of Dibrugarh district: An endemic goiter region of Assam. *Indian J. Physiol. Pharmacol.* 41 (2), 167–170.
- Thomas, M.L., Anast, C.S., Forte, L.R., 1981. Regulation of calcium homeostasis in the fetal and neonatal rat. *Am. J. Physiol.* 240, E367–E372.
- Varas, S.M., Muñoz, E.M., Hapon, M.B., Aguilera Merlo, C.I., Giménez, M.S., 2002. Hyperthyroidism and production of precocious involution in the mammary glands of lactating rats. *Reproduction* 124, 691–702.
- Venditti, P., Balestrieri, M., Di Meo, S., De Leo, T.J., 1997. Effect of thyroid state on lipid peroxidation, antioxidant defences and susceptibility to oxidative stress in rat tissues. *Endocrinology* 138, 151–157.
- Wasniewska, M., De Luca, F., Cassio, A., Oggiano, N., Gianino, P., Delvecchio, M., Aiuzzi, R., Stoppioni, V., Lombardo, F., Messina, M.F., Valenzise, M., Arrigo, T., 2003. In congenital hypothyroidism bone maturation at birth may be a predictive factor of psychomotor development during the first year of life irrespective of other variables related to treatment. *Eur. J. Endocrinol.* 149, 1–6.
- Wassef, N.W., Hassan, N.H.A., 1993. Teratogenic and cytogenetic effects of carbimazole on pregnant mice and their fetuses. *Egypt. J. Med. Sci.* 14, 543–559.
- Wong, C.C., Leung, M.S., 2001. Effects of neonatal hypothyroidism on the expressions of growth cone proteins and axon guidance molecules related genes in the hippocampus. *Mol. Cell. Endocrinol.* 184 (1–2), 143–150.
- Wong, A.O., Zhou, H., Jiang, Y., Ko, W.K., 2006. Feedback regulation of growth hormone synthesis and secretion in fish and the emerging concept of intrapituitary feedback loop. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.* 144 (3), 284–305.

- Zhang, L., Cooper-Kuhn, C.M., Nannmark, U., Blomgren, K., Kuhn, H.G., 2010. Stimulatory effects of thyroid hormone on brain angiogenesis in vivo and in vitro. *J. Cereb. Blood Flow Metab.* 30(2), 323–335 (Epub 2009 October 28).
- Zhou, Y., Wang, X., Hadley, J., Corey, S.J., Vasilatos-Younken, R., 2005. Regulation of JAK2 protein expression by chronic, pulsatile GH administration *in vivo*: a possible mechanism for ligand enhancement of signal transduction. *Gen. Comp. Endocrinol.* 144, 128–139.
- Zoeller, R.T., 2007. Environmental chemicals impacting the thyroid: targets and consequences. *Thyroid* 17 (9), 811–817.
- Zoeller, R.T., Crofton, K.M., 2005. Mode of action: Developmental thyroid hormone insufficiency-neurological abnormalities resulting from exposure to propylthiouracil. *Crit. Rev. Toxicol.* 35, 771–781.