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Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: II—The developmental pattern of neurons in relation to oxidative stress and antioxidant defense system

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ABSTRACT

Excessive concentrations of free radicals in the developing brain may lead to neurons maldevelopment and neurons damage and death. Thyroid hormones (THs) states play an important role in affecting the modulation of oxidative stress and antioxidant defense system. Thus, the objective of this study was to clarify the effect of hypothyroidism and hyperthyroidism in rat dams on the neurons development of different brain regions of their offspring at several postnatal weeks in relation to changes in the oxidative stress and antioxidant defense system. The adult female rats were administered methimazole (MMI) in drinking water (0.02% w/v) from gestation day 1 to lactation day 21 to induce hypothyroidism and exogenous thyroxine (T4) in drinking water (0.002% w/v) beside intragastric incubation of 50–-200 T4 μg/kg body weight (b. wt.) to induce hyperthyroidism. In normal female rats, the sera total thyroxine (TT4) and total triiodothyronine (TT3) levels were detectably increased at day 10 post-partum than those at day 10 of pregnancy. Free thyroxine (FT4), free triiodothyronine (FT3), thyrotropin (TSH) and growth hormone (GH) concentrations in normal offspring were elevated at first, second and third postnatal weeks in an age-dependent manner. In hypothyroid group, a marked depression was observed in sera of dam TT3 and TT4 as well as offspring FT3, FT4 and GH, while there was a significant increase in TSH level with the age progress. The reverse pattern to latter state was recorded in hyperthyroid group. Concomitantly, in control offspring, the rate of neuron development in both cerebellar and cerebral cortex was increased in its density and complexity with age progress. This development may depend, largely, on THs state. Both maternal hypothyroidism and hyperthyroidism caused severe growth retardation in neurons of these regions of their offspring from the first to third weeks. Additionally, in normal offspring, seven antioxidant enzymes, four non-enzymatic antioxidants and one oxidative stress marker (lipid peroxidation, LPO) followed a synchronized course of alterations in cerebrum, cerebellum and medulla oblongata. In both thyroid states, the oxidative damage has been demonstrated by the increased LPO and inhibition of enzymatic and non-enzymatic antioxidants in most examined ages and brain regions. These disturbances in the antioxidant defense system led to deterioration in the neuronal maturation and development. In conclusion, it can be suggested that the maldevelopment of neurons and dendrites in different brain regions of offspring of hypothyroid and hyperthyroid mother rat dams may be attributed, at least in part, to the excess oxidative stress and deteriorated antioxidant defense system in such conditions.

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1. Introduction

Thyroid hormones (THs) are essential for the proper development of numerous tissues, notably the brain (Carageorgiou et al., 2007; Ahmed et al., 2008; Visser et al., 2008; Zoeller, 2008; Ahmed et al., 2010). Also, several reports are listed on the harmful effect of TH deficiency during the development (Koibuchi, 2006; Carageorgiou et al., 2007; Stoica et al., 2007; Ahmed et al., 2008; Hasebe et al., 2008). A number of other authors (Higuchi et al., 2001; Jaeggi and Roman, 2006; Ahmed et al., 2008, 2010) recorded that hyperthyroidism is accompanied by THs increment during the development. Neonatal hyperthyroidism in rats (Varma and Crawford, 1979) results in permanent decrease in pituitary reserve of thyrotropin (TSH) secretion and permanent imprinting regarding

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growth and thyroidal development and thus, neonatal period is critical for thyroidal development. Furthermore, hypo- or hyperthyroidism affects the maturation of the central nervous system (CNS) and causes irreversible dysfunction of the brain if not corrected shortly after the birth (Wong and Leung, 2001). This late effect of neonatal hypo- or hyperthyroidism on the CNS is probably leading to defective neuronal circuit formation (Ahmed et al., 2008). In addition, both hypo- and hyperthyroidism are known to affect directly or indirectly the proliferation, apoptosis and differentiation of several neuronal and glial cell types during the postnatal brain development (Oppenheimer and Schwartz, 1997; Ahmed et al., 2008).

Generally, hyperthyroid animals appear to have a shorter life and, at advanced age, show a myelin deficiency (Pasquini and Adamo, 1994). They suggested that these changes may be due to the damage produced by the oxidative stress generated by an excess of THs. Furthermore, Yavuz et al. (2008) reported that TSH suppression therapy with L-thyroxine (L-T4) leading to subclinical hyperthyroidism may cause increased oxidative stress in euthyroid nodular goiter patients. On the other hand, Abalovich et al. (2003) and Suchetha Kumari et al. (2011) indicated an increase in oxidative stress and a decrease in the antioxidant system markers in hyperthyroid patients with Graves' disease. In addition, rat hypothyroidism was found to be associated with marked oxidative stress, one of the earliest manifestations of which was a decline in the level of glutathione (Rahaman et al., 2001). Therefore, the objective of this study was designed to assess the probable alterations induced by maternal hypo- and hyperthyroidism on the neuronal development of cerebellar and cerebral cortex and on the balance between antioxidant defense systems and oxidative stress markers in distinct developmental stages of rat offspring.

2. Materials and methods

2.1. Experimental animals

The present study was carried out on white albino rat (*Rattus norvegicus*); 46 mature virgin females weighting about 170–190 g and 11 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 (Zoology Department, Faculty of Science, Beni-Suef University, Egypt) 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 cages with well ventilation at normal atmospheric temperature (23 ± 2 °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 exposed to constant daily light/dark periods of 12 h (h) each (lights on at 06:00 h) and 50 ± 5% relative humidity. Generally, the protocol follows the general guidelines of animal care (Ernest et al., 1993). All efforts were made to minimize the number of animals used and their suffering. Mating was induced by housing proesterous 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 first day of pregnancy [gestation day (GD) 1] to the first 3 weeks of lactation period [lactation day (LD) 21] were divided into three groups as follows:

- Hypothyroid group: 15 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).
- Hyperthyroid group: Further 15 rats were rendered hyperthyroid by exogenous thyroxine (T4) (Eltroxine tablets; GlaxoWellcome, Germany) intragastric administration in increasing doses beginning from 50 μg to reach 200 μg/Kg body weight (b. wt.) beside adding 0.002% (w/v) T4 to the drinking water (Guerrero et al., 1999; Ahmed, 2006) directly after mating (GD 1–LD 21).
- Control group: 16 control rats received tap water.

The mother sera (six 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 states. The blood samples were taken from optic vein and centrifuged at 3000 round per minute (r.p.m.) for 30 min (min). The clear, non-hemolyzed supernatant sera were quickly removed, divided into three portions for each individual animal, and kept at −30 °C until use.

After the pregnancy, the decapitation of normal, hypothyroid and hyperthyroid offspring was done at the end of the first, second and third postnatal weeks under mild diethyl ether anaesthesia. The blood samples were taken from jugular vein, centrifuged and kept at −30 °C until use. For investigation of the neurons development and dendrites arborization, cerebella and cerebra were rapidly excised and fixed in 4:1 mixture of 5% potassium dichromate and 40% formalin before further processing and staining with Golgi-Copsch stain. On the other hand, the cerebrum, cerebellum and medulla oblongata (MO) of these offsprings were quickly removed, separated and homogenized by using a Teflon homogenizer (Glas-Col, Terre Haute in USA) and kept in deep freezer at −30 °C until use.

2.3. The radioimmunoassay examinations

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 offspring were determined (quantitative measurement) in Diabetic Endocrine Metabolic Pediatric Unit, Center for Social and Preventive Medicine, New Children Hospital, Faculty of Medicine, Cairo University. The kit was obtained from Calbiotech INC (CBI), USA. The procedures were estimated according to the method of Thakur et al. (1997), Maes et al. (1997), Larsen (1982), Smals et al. (1981), Mandel et al. (1993) and Reutens (1995), respectively.

2.4. The biochemical examinations

Determination of oxidative stress markers and antioxidant defense system were estimated in our laboratory. The ascorbic acid (vitamin C), α-tocopherol (vitamin E), total thiol (t-SH), glutathione (GSH) and lipid peroxidation (LPO) process were measured according to the method of Kyaw (1978), Hawk et al. (1954), Koster et al. (1986), Beutler et al. (1963) and Preuss et al. (1998), respectively. Also, the glutathione reductase (GSSGR), glutathione-S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), peroxidase (PO) and polyphenol oxidase (PPO) activities were estimated according to the method of Goldberg and Spooner (1983), Mannervik and Guthenberg (1981), Pinto and Bartley (1989), Marklund and Marklund (1974), Cohen et al. (1970), Kar and Mishra (1976) and Kar and Mishra (1976), respectively.

2.5. Golgi-Copsch stain

The Golgi-Copsch staining was performed according to Tömböl (1967) and Ahmed et al. (2007). The cerebella and cerebra fixed in 4:1 mixture of 5% potassium dichromate and 40% formalin for 4 days were transferred to 3.6% potassium dichromate for another 4 days. The tissues were washed with 0.75% silver nitrate and then placed in the same solution for 4 days. The last two steps were repeated once. The slices were dehydrated then placed in xylene for 20 min. Embedding process was made in paraffin wax. Thereafter, serial sections were made at 50 μm to show the cortex neurons of both regions. The dewaxed process was carried out by using xylene. The sections were mounted in Canada balsm.

2.6. Statistical analysis

The data are analyzed using one-way analysis of variance (ANOVA) (PC-STAT, 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. The data are presented as mean ±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.

3. Results

3.1. Serum-hormonal levels (Table 1 and Figs. 1–3)

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

3.1.1. Maternal total thyroxine (TT4) and total triiodothyronine (TT3) concentrations (Fig. 1)

Fig. 1 showed higher serum TT4 and TT3 of adult female rats at day 10 post-partum than those at gestational day 10 in control

Table 1
Effect of thyroid status on ascorbic acid (vitamin C, μg/100 mg), α-tocopherol (Vitamin E, nmol/100 mg), total thiol (t-SH, nmol/100 mg), glutathione (GSH, nmol/100 mg) and lipid peroxidation process (LPO, nmol MDA/100 mg/h) in different brain regions at various postnatal ages of rat newborns.

Periods	Status	Ascorbic acid			α-Tocopherol			t-SH		
		CR	CB	MO	CR	CB	MO	CR	CB	MO
1 Week	Control	37.654 ± 0.559^{c}	30.809 ± 0.581^{c}	35.655 ± 0.336^{c}	184.408 ± 0.893^{c}	202.355 ± 3.109^{c}	189.554 ± 0.603^{c}	80.691 ± 2.137^{c}	61.071 ± 2.119^{c}	89.955 ± 2.004^{c}
	Hypothyroid	26.101 ± 0.850^{e}	$20.051 \pm 0.424^{d-e}$	24.104 ± 1.029^{d}	169.150 ± 0.423^{e}	155.011 ± 1.208^{d}	159.850 ± 0.648^{d}	66.824 ± 1.641^{d}	44.468 ± 2.558^{d}	68.883 ± 3.627^{e}
	Hyperthyroid	31.300 ± 0.269^{d}	21.006 ± 0.537^{d}	22.156 ± 0.201^{e}	173.551 ± 1.051^{d}	157.003 ± 1.655^{d}	160.901 ± 0.850^{d}	69.380 ± 2.447^{d}	$38.462 \pm 2.439^{d-e}$	82.141 ± 2.220^{d}
2 Week	Control	45.459 ± 1.811^{b}	42.057 ± 0.559^{b}	47.357 ± 0.828^{b}	220.453 ± 0.962^{b}	247.550 ± 1.186^{b}	218.855 ± 0.872^{b}	97.258 ± 3.062^{b}	78.655 ± 2.432^{b}	117.057 ± 1.929^{b}
	Hypothyroid	19.152 ± 0.067^{f}	17.805 ± 0.402^{f}	16.406 ± 0.223^{f}	155.054 ± 1.453^{g}	143.351 ± 1.140^{e}	137.804 ± 1.029^{f}	46.893 ± 2.377^{e}	33.209 ± 2.194^{e}	49.208 ± 2.163^{g}
	Hyperthyroid	18.854 ± 0.111^{f}	$18.850 \pm 0.022^{e-f}$	$15.055 \pm 0.111^{f-g}$	165.157 ± 0.774^{f}	119.502 ± 0.367^{g}	149.053 ± 0.961^{e}	51.714 ± 1.743^{e}	24.313 ± 0.823^{f}	59.424 ± 2.416^{f}
3 Week	Control	68.055 ± 0.201^{a}	56.554 ± 1.006^{a}	50.054 ± 0.917^{a}	298.759 ± 0.960^{a}	282.600 ± 0.759^{a}	262.504 ± 0.849^{a}	124.507 ± 2.090^{a}	102.533 ± 3.306^{a}	146.463 ± 3.778^{a}
	Hypothyroid	14.600 ± 0.626^{g}	11.351 ± 0.559^{h}	13.751 ± 0.469^{g}	131.754 ± 0.648^{i}	133.907 ± 1.654^{f}	122.359 ± 0.782^{g}	29.722 ± 1.293^{f}	$21.062 \pm 2.029^{f-g}$	30.778 ± 1.758^{i}
	Hyperthyroid	16.056 ± 0.247^{g}	13.652 ± 0.558^{g}	11.950 ± 0.111^{h}	151.705 ± 0.403^{h}	110.150 ± 1.140^{h}	106.406 ± 1.744^{h}	34.544 ± 2.092^{f}	17.496 ± 0.950^{g}	40.132 ± 2.548^{h}
LSD at 5% level		2.130	1.645	1.676	2.567	4.443	2.827	6.218	6.409	7.467
LSD at 1% level		2.869	2.215	2.257	3.457	5.984	3.807	8.374	8.631	10.056

		GSH			LPO		
		CR	CB	MO	CR	CB	MO
1 Week	Control	26.699 ± 1.431^{c}	20.802 ± 0.403^{c}	29.267 ± 1.552^{c}	26.503 ± 0.773^{e}	28.611 ± 0.050^{f}	37.459 ± 0.251^{g}
	Hypothyroid	18.438 ± 0.640^{d}	17.346 ± 1.047^{d}	22.141 ± 0.933^{d}	30.947 ± 0.483^{d}	37.244 ± 0.953^{e}	43.275 ± 1.063^{f}
	Hyperthyroid	16.111 ± 0.379^{d}	12.422 ± 1.032^{e}	22.472 ± 2.284^{d}	38.297 ± 2.074^{c}	40.908 ± 1.830^{e}	58.670 ± 2.403^{d}
2 Week	Control	39.065 ± 1.879^{b}	28.345 ± 1.560^{b}	41.859 ± 2.088^{b}	30.586 ± 0.341^{d}	31.564 ± 0.453^{f}	45.015 ± 0.759^{f}
	Hypothyroid	12.871 ± 0.093^{e}	11.411 ± 0.728^{e}	16.988 ± 0.437^{e}	38.330 ± 1.194^{c}	51.974 ± 2.220^{d}	56.690 ± 1.978^{d}
	Hyperthyroid	12.579 ± 0.803^{e}	11.639 ± 0.686^{e}	$15.310 \pm 0.598^{e-f}$	52.942 ± 1.849^{b}	60.950 ± 2.736^{c}	81.804 ± 1.340^{b}
3 Week	Control	54.946 ± 1.644^{a}	42.038 ± 1.324^{a}	55.808 ± 2.072^{a}	35.076 ± 0.059^{c}	40.177 ± 0.393^{e}	50.144 ± 0.079^{e}
	Hypothyroid	8.140 ± 0.439^{f}	6.638 ± 0.791^{f}	11.963 ± 0.608^{f}	51.817 ± 1.179^{b}	66.339 ± 1.930^{b}	76.988 ± 0.914^{c}
	Hyperthyroid	8.052 ± 0.940^{f}	6.703 ± 0.467^{f}	6.885 ± 0.813^{g}	71.938 ± 1.618^{a}	77.990 ± 2.139^{a}	99.532 ± 1.600^{a}
LSD at 5% level		3.129	2.782	4.166	3.615	4.844	3.928
LSD at 1% level		4.213	3.747	5.611	4.869	6.524	5.289

CR is Cerebrum, CB is Cerebellum and MO is Medulla Oblongata. Data are expressed as mean ±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.

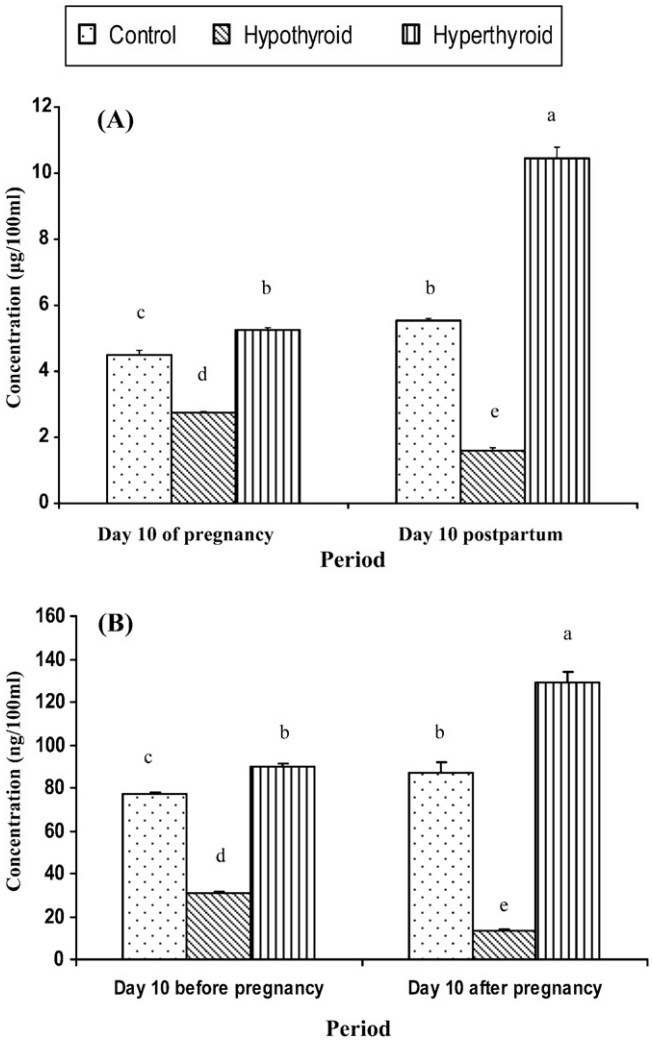

Fig. 1. Effect of thyroid status on total thyroxine (TT4) (A) and total triiodothyronine (TT3) (B) concentrations in serum of pregnant and lactating female rats. TT4: *F*-probability: $P < 0.001$; LSD at the 5% level: 0.289; LSD at the 1%: 0.389. TT3: *F*-probability: $P < 0.001$; LSD at the 5% level: 1.900; LSD at the 1%: 2.560.

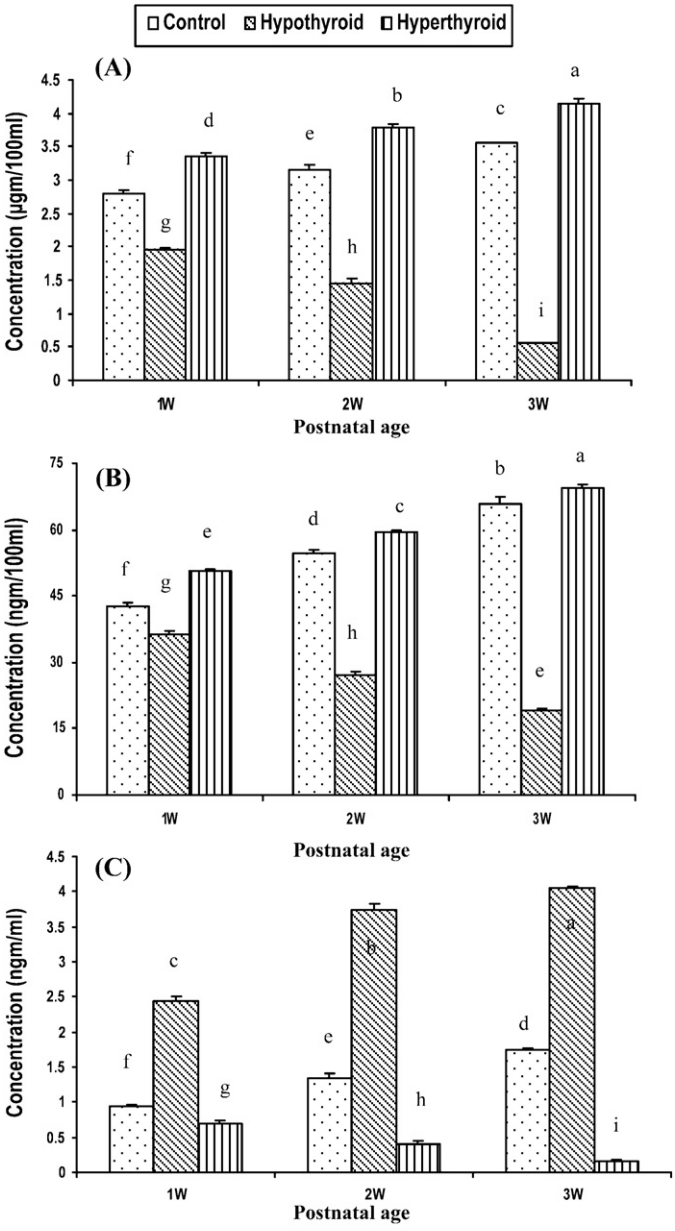

Fig. 2. Effect of thyroid status on free thyroxine (FT4) (A), free triiodothyronine (FT3) (B) and thyrotropin (TSH) (C) concentrations in serum of rat newborns. (W; week). *F*-probability: $P < 0.001$. LSD at the 5% and 1% levels are 0.148 and 0.198 for FT4; 2.492 and 3.355 for FT3; 0.134 and 0.181 for TSH, respectively.

group. Generally, administration of MMI to female rats from GD 1 to LD 21 resulted in a marked decrease (LSD; $P < 0.01$) of serum TT4 and TT3 levels (characteristic of hypothyroidism); at day 10 during the pregnancy, 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 (Table 1). Conversely, the administration of exogenous T4 during the same previous periods exhibited the reverse pattern of changes; serum TT4 and TT3 levels increased significantly (LSD; $P < 0.01$) at day 10 during pregnancy and after birth.

Considering one-way ANOVA analysis of TT4 and TT3, the general effect between groups was very highly significant ($P < 0.001$) throughout the experiment.

3.1.2. Free thyroxine (FT4), free triiodothyronine (FT3), thyrotropin (TSH) and growth hormone (GH) concentrations in offspring (Figs. 2 and 3)

The effects of thyroid dysfunction, hypo- and hyperthyroidism, on serum FT4, FT3, TSH and GH levels at the end of the first, second and third weeks after birth of rat offspring, were allotted in Figs. 2 and 3 and Table 1. In normal rat offspring, the concentrations of these parameters 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 offspring of hypothyroid mothers whose serum TSH levels were significantly elevated (LSD; $P < 0.01$). However, FT4, FT3 and GH levels in the offspring 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 first to third postnatal weeks as compared with the corresponding controls (Figs. 2 and 3). Moreover, at the end of the third week, TSH levels in hyperthyroid group were very low as compared to the levels in the age-matched normal controls (0.150 ± 0.022 vs. 1.750 ± 0.021).

With regards one-way ANOVA of FT4, FT3, TSH and GH, the general effect between groups was found to be very highly significant ($P < 0.001$) throughout the experiment.

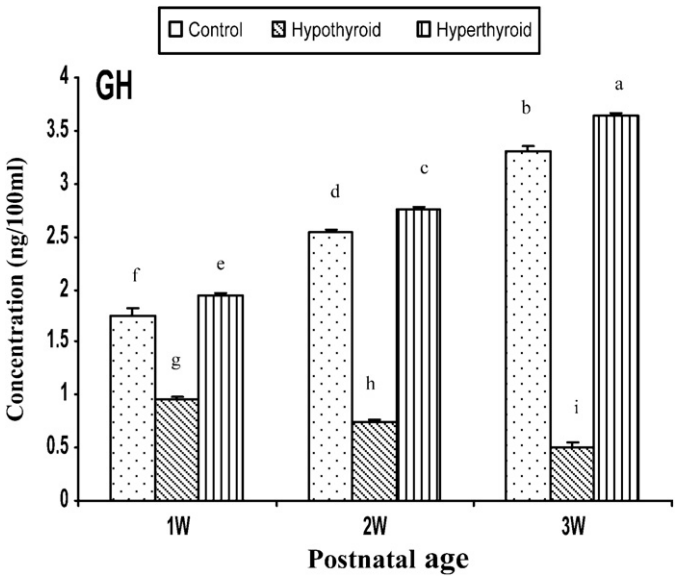

Fig. 3. Effect of thyroid status on growth hormone (GH) concentration in serum of rat newborns. (W; week). *F*-probability: $P < 0.001$; LSD at the 5% level: 0.103; LSD at the 1% level: 0139.

3.2. Oxidative stress markers and antioxidant defense system in different brain regions of offspring (Tables 1 and 2)

3.2.1. Ascorbic acid (vitamin C), α-tocopherol (vitamin E), total thiol (t-SH) and glutathione (GSH) concentrations (Table 1)

It is clear from Table 2 that the normal values of these parameters were markedly increased in an age-dependent manner in all tested brain regions to reach maximum values at the end of the third postnatal week. The concentrations of these parameters were affected by both treatments in respect to control group. In all studied regions and periods of both treated groups, the concentrations of vitamin C and E were decreased (LSD; $P < 0.01$) in comparison with their corresponding controls. Also, in both treated groups, there were a highly significant decreases (LSD; $P < 0.01$) in the t-SH and GSH concentrations at the end of the first week in all investigated regions except in MO of hyperthyroid group where the decrease in t-SH was significant (LSD; $P < 0.05$) and in cerebellum of hypothyroid group where the decrease in GSH was also significant. As the age progressed to second week in both treated groups, a significant reduction (LSD; $P < 0.01$) in the concentrations of t-SH and GSH in all investigated regions was observed as compared with the corresponding controls. Generally, a highly significant impact (LSD; $P < 0.01$) in preserving the level of these parameters was observed at the end of the third week in both treated groups in all tested regions (Table 1).

Considering one-way ANOVA of vitamin C and E, t-SH and GSH the general effect between groups was very highly significant ($P < 0.001$) (Table 1) in all tested regions.

3.2.2. Lipid peroxidation (LPO) (Table 1)

The results of LPO (thiobarbituric acid reacting substances; TBARS) obtained under the conditions described in the experimental part were represented in Table 1. Additionally, the data of the normal rat offspring indicated a gradual increase in TBARS concentration in all investigated regions of rat offspring with the age progress. As expected, along the duration of the experiment from the first to third weeks, in both hypo- and hyperthyroid groups, a highly significant increase (LSD; $P < 0.01$) in the TBARS concentration was found in all tested regions in comparison with the control one except in cerebrum of hypothyroid group, this increase was significant only (LSD; $P < 0.05$) at the end of the first week (Table 1). Interestingly, hyperthyroid offspring had a higher TBARS level than hypothyroid values. Notably, in both treated groups, the level of TBARS showed its highest profile (LSD; $P < 0.01$) at the end of the third postnatal week in all studied brain regions as compared to the levels in the age-matched normal controls (Table 1).

Regarding one-way ANOVA, the general effect between groups was very highly significant ($P < 0.001$) in all tested regions (Table 1).

3.2.3. Glutathione reductase (GSSGR), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities (Table 2)

Table 2 showed that the activities of GSSGR, GST, GPx and SOD of normal rat offspring exhibited a stepwisely increased with the age progress. On the other hand, this behavioral pattern was disrupted in both treated groups which led to a significant suppression of these activities in all tested regions and all ages except at the end of the first week, in cerebrum and MO, for the activity of GSSGR and SOD in hyperthyroid group only, which showed an increase as compared with the control group. In both treated groups, the most potent decrease (LSD; $P < 0.01$) of these enzymes activities was recorded in all tested regions at the end of the third week (Table 2). With regard to the one-way ANOVA of these parameters, the general effect between groups was very highly significant ($P < 0.001$) (Table 2) in all investigated regions throughout the experiment.

3.2.4. Catalase (CAT), peroxidase (PO) and polyphenol oxidase (PPO) activities (Table 2)

Similar to the previous enzymes, Table 2 depicted that CAT, PO and PPO activities in normal offspring were markedly increased in an age-dependent manner in all tested regions. Also, in both treated groups, the enzyme activities showed fluctuated changes. In cerebellum and MO, the CAT activity profoundly decreased (LSD; $P < 0.01$) in both treated groups at all tested ages except at the end of the first week of hyperthyroid group only. Though the CAT activity, in cerebrum of hypothyroid group, was enormously increased (LSD; $P < 0.01$) at the end of the second week, it was detectably diminished (LSD; $P < 0.01$) at other ages in comparison with the values of the corresponding controls. Furthermore, the CAT activity was clearly increased (LSD; $P < 0.01$) at the end of the first and second weeks and depressed (LSD; $P < 0.01$) at the end of the experiment in cerebrum of hyperthyroid group (Table 2). Moreover, both treated offsprings exhibited an obvious decrease (LSD; $P < 0.01$) of the PO activity in all investigated regions during the studied developmental ages except in hyperthyroid group at the end of the first week in all tested regions and at the end of the second week in cerebellum only when compared to the corresponding normal controls (Table 2). On the other hand, compared to controls, hypothyroid group was characterized by a highly significant depletion (LSD; $P < 0.01$) of the PPO activity in all investigated regions at the first two tested weeks. On the other hand, in all studied regions, even though the activity of PPO in hyperthyroid group was increased significantly (LSD; $P < 0.01$) at the end of the first postnatal week, this activity was profoundly depressed (LSD; $P < 0.01$) at the end of the second week. With the age progress to the end of the third postnatal week, the recorded activity of PPO, in all tested regions, was more deteriorated (LSD; $P < 0.01$) in both treated groups than the control activity (Table 2).

The analysis of one-way ANOVA of CAT, PO and PPO recorded that the general effect between groups was very highly significant ($P < 0.001$) throughout the experiment (Table 2).

3.3. The cerebellar neurons of offspring as shown by Golgi-Copsch stain (Figs. 4–6)

In normal and both hypo- and hyperthyroid groups, five types of neurons in layers of cerebellar cortex (gray matter) were recorded.

Table 2
Effect of thyroid status on glutathione reductase (GSSGR, mU/100 mg), glutathione-S-transferase (GST, U/100 mg), glutathione peroxidase (GPx, mU/100 mg), superoxide dismutase (SOD, mU/100 mg), catalase (CAT, k.100), peroxidase (PO, mU/g) and polyphenol oxidase (PPO, mU/g) in different brain regions at various postnatal ages of rat newborns.

Periods	Status	GSSGR			GST			GPx			SOD		
		CR	CB	MO	CR	CB	MO	CR	CB	MO	CR	CB	MO
1 Week	Control	57.847±2.997^{c}	49.124±1.401^{c}	77.491±0.868^{c}	6.291±0.023^{c}	7.187±0.042^{c}	8.109±0.077^{c}	95.333±0.332^{c}	92.297±0.304^{c}	106.709±1.452^{c}	35.759±0.469^{d}	46.008±2.147^{c}	34.957±1.274^{d}
	Hypothyroid	31.016±1.701^{d}	27.211±2.123^{e}	46.740±0.650^{e}	5.328±0.044^{d}	5.740±0.023^{e}	7.317±0.081^{d}	84.021±1.513^{d-e}	73.414±0.577^{e}	84.710±0.422^{e}	19.501±1.029^{e}	29.350±1.007^{e}	25.500±0.716^{e}
	Hyperthyroid	69.459±1.319^{b}	37.223±2.342^{d}	87.377±0.819^{b}	5.348±0.020^{d}	6.468±0.056^{d}	5.510±0.293^{e}	87.560±1.090^{d}	87.890±1.060^{d}	95.274±1.029^{d}	50.854±2.438^{c}	36.264±1.096^{d}	39.752±0.424^{c}
2 Week	Control	71.904±2.964^{b}	61.899±1.111^{b}	89.978±2.092^{b}	7.833±0.102^{b}	8.189±0.044^{b}	8.785±0.151^{b}	109.539±0.422^{b}	120.304±1.332^{b}	130.457±0.184^{b}	56.358±0.649^{b}	75.766±0.828^{b}	53.151±0.917^{b}
	Hypothyroid	22.518±0.819^{e}	16.486±1.006^{g}	33.107±1.480^{f}	4.515±0.049^{e}	4.499±0.187^{f}	5.827±0.053^{e}	73.622±0.788^{f}	58.322±1.089^{g}	72.801±0.849^{f}	13.600±0.448^{f}	20.054±0.157^{f}	17.653±0.336^{f}
	Hyperthyroid	53.904±2.883^{c}	24.816±1.598^{e-f}	68.118±3.083^{d}	4.431±0.160^{e}	5.921±0.016^{e}	4.051±0.083^{f}	80.107±0.541^{e}	75.913±0.724^{e}	82.272±1.270^{e}	33.650±0.782^{d}	21.852±0.111^{f}	17.801±0.939^{f}
3 Week	Control	92.327±1.092^{a}	71.910±2.384^{a}	107.046±1.948^{a}	9.536±0.351^{a}	11.055±0.344^{a}	10.765±0.160^{a}	121.048±0.391^{a}	129.369±1.994^{a}	135.325±1.269^{a}	98.154±1.900^{a}	101.767±1.186^{a}	93.758±1.632^{a}
	Hypothyroid	14.405±1.292^{f}	10.261±0.212^{h}	17.662±2.737^{g}	3.241±0.019^{f}	2.937±0.074^{g}	4.358±0.295^{f}	42.410±0.453^{h}	44.380±1.632^{h}	54.600±1.663^{h}	10.106±0.313^{g}	8.840±0.469^{h}	8.704±0.804^{g}
	Hyperthyroid	35.873±2.020^{d}	21.486±2.793^{f-g}	41.626±2.590^{e}	2.265±0.021^{g}	4.312±0.372^{f}	3.177±0.135^{g}	59.881±1.243^{g}	68.684±1.847^{f}	66.910±0.396^{g}	18.001±0.983^{e}	12.251±1.140^{g}	9.505±0.269^{g}
LSD at 5% level		6.406	5.299	5.768	0.392	0.532	0.494	4.576	3.739	3.622	3.488	3.114	2.642
LSD at 1% level		8.627	7.136	7.768	0.528	0.716	0.665	6.162	5.036	4.878	4.697	4.194	3.559

Periods	Status	CAT			PO			PPO		
		CR	CB	MO	CR	CB	MO	CR	CB	MO
1 Week	Control	7.396±0.234^{f}	8.112±0.350^{d}	10.216±0.251^{d}	108.387±3.416^{d}	92.717±0.686^{e}	136.357±1.792^{d}	64.675±1.309^{d}	70.687±0.944^{e}	71.111±3.257^{e}
	Hypothyroid	5.904±0.049^{g}	5.041±0.022^{g}	7.135±0.260^{g}	97.765±0.610^{e}	80.777±0.796^{f}	102.195±1.123^{f}	56.923±0.967^{e}	61.998±1.250^{f}	59.243±0.701^{f}
	Hyperthyroid	8.784±0.152^{d-e}	9.944±0.066^{b}	11.229±0.132^{c}	121.257±1.613^{c}	123.617±1.460^{b}	149.695±1.076^{c}	80.210±0.146^{b}	83.960±0.713^{c}	87.725±2.076^{c}
2 Week	Control	9.336±0.298^{d}	10.143±0.321^{b}	12.967±0.428^{b}	146.672±3.939^{b}	102.765±1.191^{d}	158.875±0.674^{b}	73.347±1.350^{c}	88.757±1.061^{b}	98.232±0.507^{b}
	Hypothyroid	10.457±0.208^{c}	7.228±0.261^{e}	9.982±0.163^{d-e}	79.468±2.680^{f}	71.092±1.607^{g}	88.744±1.703^{h}	42.308±1.816^{f}	51.716±0.369^{g}	51.484±0.258^{g}
	Hyperthyroid	11.061±0.162^{b}	9.220±0.058^{c}	10.076±0.094^{d-e}	103.675±1.860^{d-e}	133.350±1.089^{a}	121.042±1.912^{e}	67.112±0.509^{d}	75.657±0.407^{d}	80.253±0.737^{d}
3 Week	Control	13.826±0.177^{a}	12.214±0.211^{a}	16.115±0.306^{a}	164.645±0.930^{a}	121.193±1.584^{b}	176.475±0.407^{a}	86.519±2.763^{a}	107.932±4.026^{a}	113.625±2.268^{a}
	Hypothyroid	8.504±0.118^{e}	6.297±0.090^{f}	8.582±0.138^{f}	66.853±1.460^{g}	57.403±1.534^{h}	73.803±1.700^{i}	30.025±0.950^{g}	45.290±0.766^{h}	36.913±1.712^{h}
	Hyperthyroid	11.524±0.308^{b}	8.331±0.156^{d}	9.508±0.214^{e}	82.378±2.157^{f}	108.455±1.758^{c}	96.029±1.084^{g}	58.210±0.944^{e}	65.582±1.901^{f}	72.327±1.008^{e}
LSD at 5% level		0.592	0.590	0.697	6.700	3.895	3.955	4.023	4.794	4.847
LSD at 1% level		0.798	0.797	0.939	9.023	5.245	5.326	5.418	6.457	6.527

CR is Cerebrum, CB is Cerebellum and MO is Medulla Oblongata. Data are expressed as mean ±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.

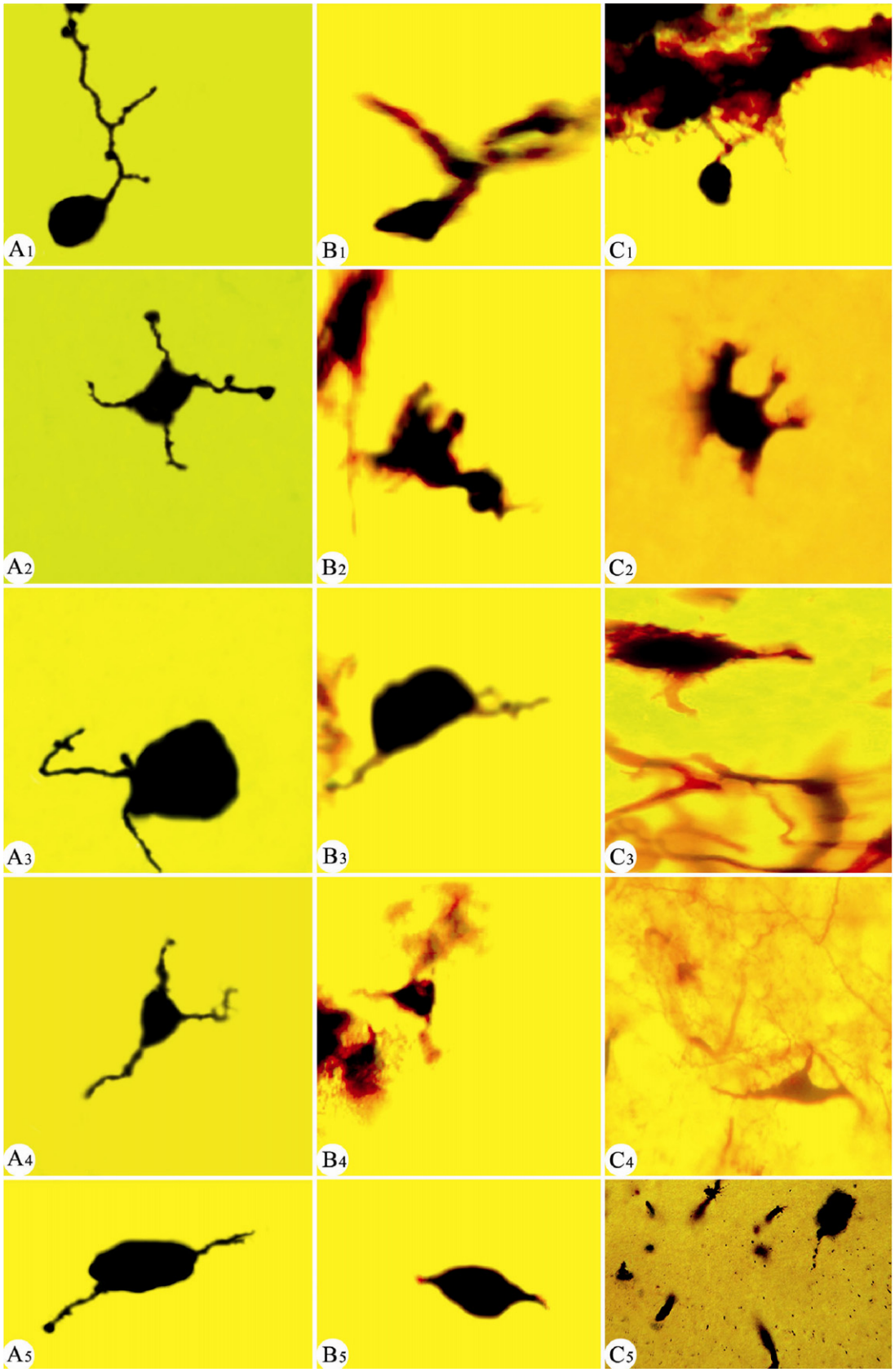

Fig. 4. Sagittal sections in the cerebellar cortex of normal, hypothyroid and hyperthyroid rat newborns, at the end of the first postnatal week, showing the following neurons: Purkinje cells (A_1: Normal; B_1: Hypothyroid; C_1: Hyperthyroid). Stellate cells (A_2: Normal; B_2: Hypothyroid; C_2: Hyperthyroid). Basket cells (A_3: Normal; B_3: Hypothyroid; C_3: Hyperthyroid). Golgi cells (A_4: Normal; B_4: Hypothyroid; C_4: Hyperthyroid). Granule cells (A_5: Normal; B_5: Hypothyroid; C_5: Hyperthyroid). (Golgi-Copsch stain, X650.)

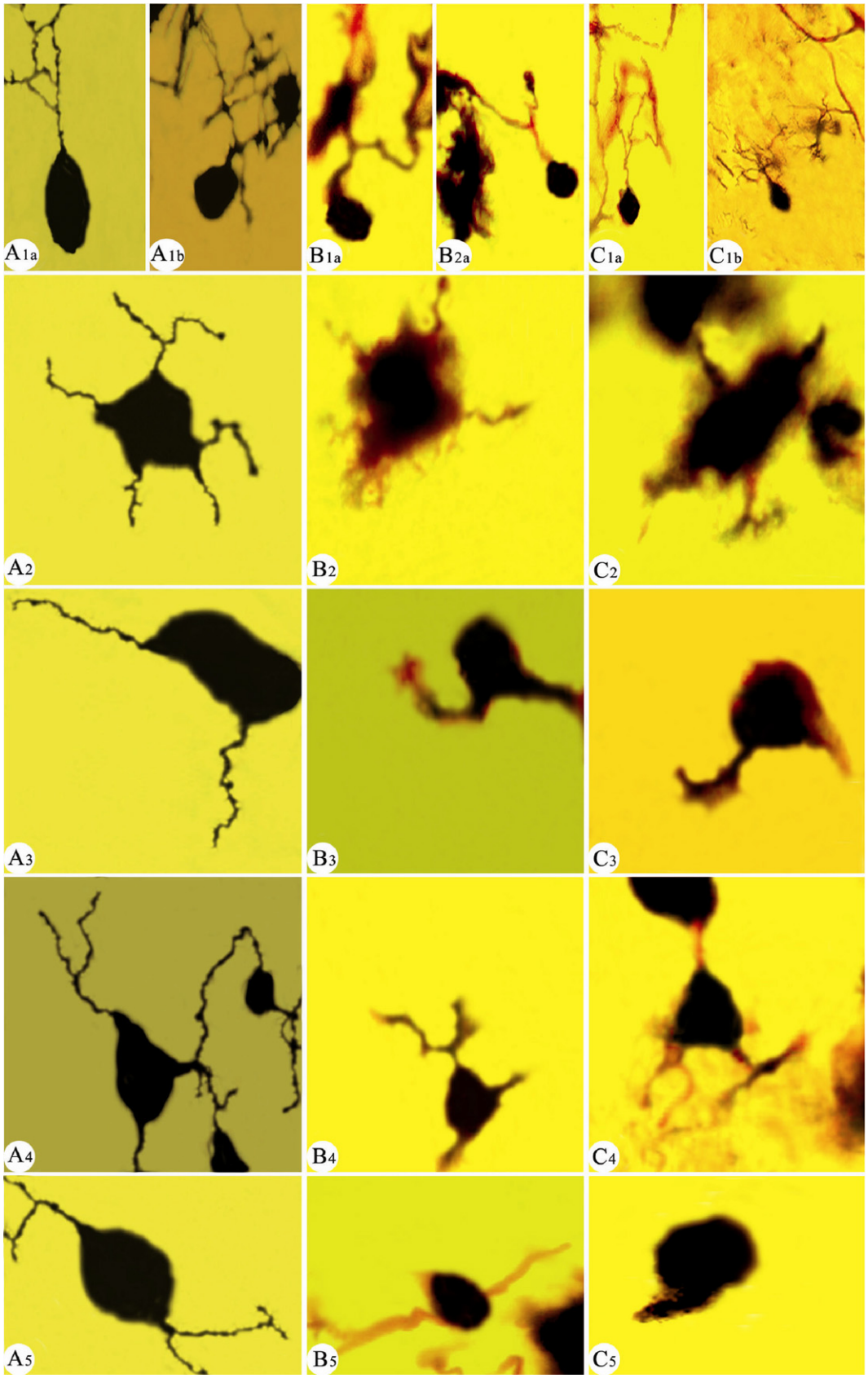

Fig. 5. Sagittal sections in the cerebellar cortex of normal, hypothyroid and hyperthyroid rat newborns, at the end of the second postnatal week, showing the following neurons: Purkinje cells (A_1: Normal; B_1: Hypothyroid; C_1: Hyperthyroid). Stellate cells (A_2: Normal; B_2: Hypothyroid; C_2: Hyperthyroid). Basket cells (A_3: Normal; B_3: Hypothyroid; C_3: Hyperthyroid). Golgi cells (A_4: Normal; B_4: Hypothyroid; C_4: Hyperthyroid). Granule cells (A_5: Normal; B_5: Hypothyroid; C_5: Hyperthyroid). (Golgi-Copsch stain, X650.)

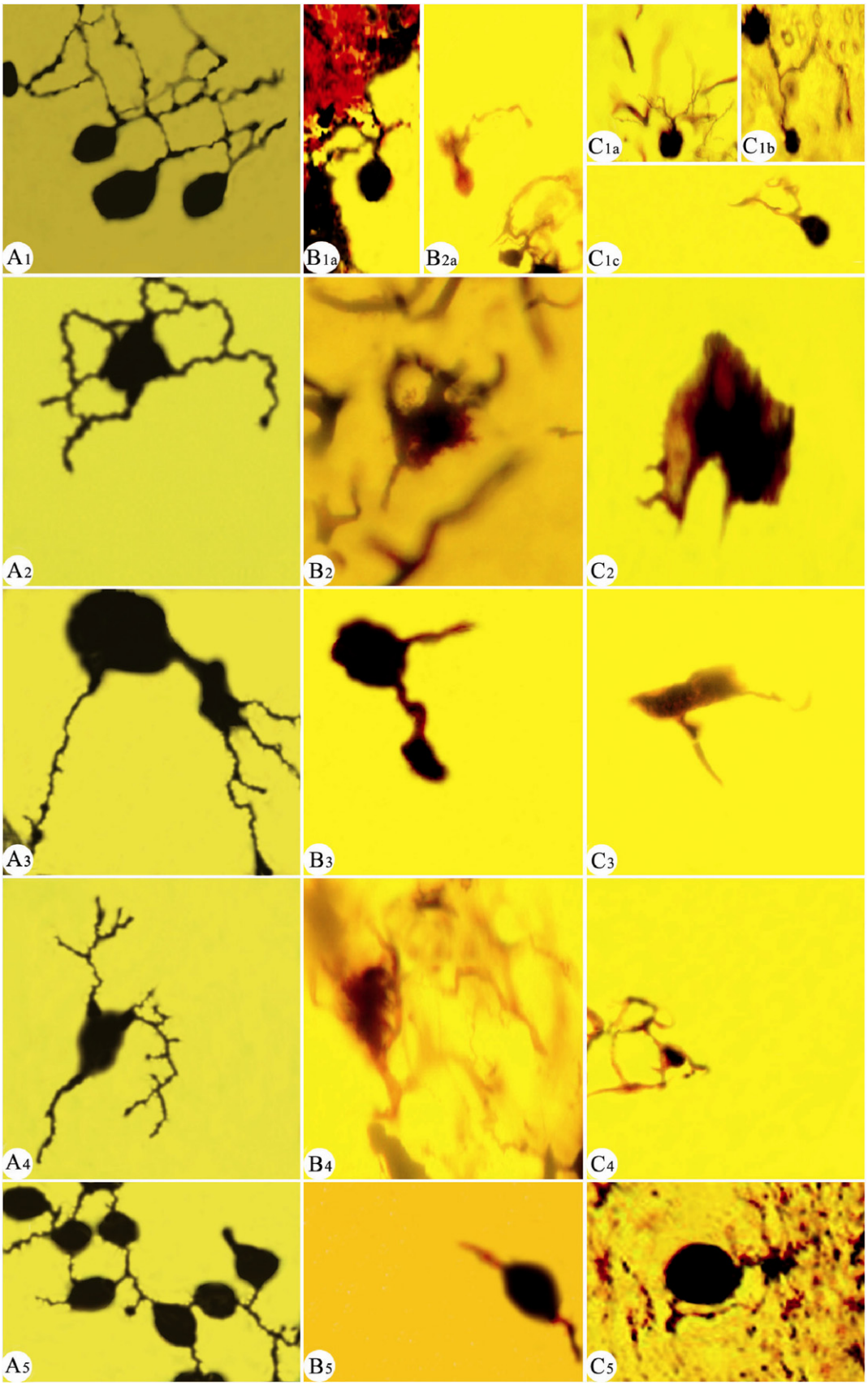

Fig. 6. Sagittal sections in the cerebellar cortex of normal, hypothyroid and hyperthyroid rat newborns, at the end of the third postnatal week, showing the following neurons: Purkinje cells (A_1: Normal; B_1: Hypothyroid; C_1: Hyperthyroid). Stellate cells (A_2: Normal; B_2: Hypothyroid; C_2: Hyperthyroid). Basket cells (A_3: Normal; B_3: Hypothyroid; C_3: Hyperthyroid). Golgi cells (A_4: Normal; B_4: Hypothyroid; C_4: Hyperthyroid). Granule cells (A_5: Normal; B_5: Hypothyroid; C_5: Hyperthyroid). (Golgi-Copsch stain, X650.)

3.3.1. The Purkinje layer (PL) (Figs. 4A$_1$–C$_1$ to 6A–C$_1$)

This layer consists of one type of neurons; the Purkinje cells (PCs). These cells in normal and both treated groups appeared possessing one main dendrite, which arise from the upper end of the cell body and extending through the ML at the end of the first postnatal week (4A$_1$–C$_1$). These primary dendrites branch repeatedly through the ML into secondary and tertiary dendrites giving a rich dendritic tree with innumerable tiny spicules at the end of the third postnatal week in normal offspring (Fig. 6A$_1$). In addition, these arborizations were confined to a plane perpendicular to the pia matter in normal offspring at the end of the second postnatal week (Fig. 5A$_1$) and reached their maximum length at the end of the third week (Fig. 6A$_1$). At the end of the first and second postnatal weeks, in normal offspring, these cells were large in size and had long dendrites when compared to those of hypo- and hyperthyroid groups (Figs. 4A$_1$–C$_1$ and 5A$_1$–C$_1$). Moreover, the latter figures showed that the dendrites of PCs had spicules and nodules in normal offspring in respect to hypo- and hyperthyroid groups. On the other hand, in both treated groups, there was some delay in the growth of the dendrites of these cells at the end of the first and second postnatal weeks (Figs. 4B$_1$ and C$_1$ and 5B$_1$ and C$_1$). Also, some important points, at the end of the third postnatal week, can be summarized (6A$_1$–C$_1$) as the following: (1) PCs of normal offspring increased in number and in their dendrites as compared to both treated ones, (2) there were few spicules on the dendrites of both treated offspring in comparison with normal ones and (3) the density of the dendritic network in both treated offsprings was lesser than that seen in the normal ones. Furthermore, some degeneration was noticed in hypo- and hyperthyroid groups at the end of the third postnatal week (6B$_1$ and C$_1$).

3.3.2. The molecular layer (ML) (Figs. 4 A$_2$, A$_3$, B$_2$, B$_3$, C$_2$ and C$_3$–6A$_2$, A$_3$, B$_2$, B$_3$, C$_2$ and C$_3$)

This layer consists of two types of neurons; the stellate and basket cells.

3.3.2.1. The stellate cells. The stellate cells were shown occupying the upper half of the ML in normal and both treated groups. At the end of the first postnatal week, these cells had thick dendrites' branches in normal offspring with the presence of some spicules and nodules on their branches when compared to their respective hypo- and hyperthyroid groups (4A$_2$–C$_2$). At other studied ages, the dendrites appeared long and thick in normal offspring and having some spicules and nodules in relation to their respective treated groups (Figs. 5A$_2$–C$_2$ and 6A$_2$–C$_2$). The branching of these dendrites form-interlacing network in normal offspring in comparison with both treated ones at the end of the third postnatal week (6A$_2$–C$_2$). Some degeneration and deformations were recorded in both treated groups during all tested periods (4B$_2$ and C$_2$, 5B$_2$ and C$_2$ and 6B$_2$ and C$_2$).

3.3.2.2. The basket cells. The basket cells in normal and both hypo- and hyperthyroid groups were found to occupy the deep region in the ML at different examined ages. They appeared rounded or oval, bipolar perikaryon and their branches moving parallel to the pial surface in normal and both treated groups at the end of the first postnatal week (4A$_3$–C$_3$). However, the dendrites of these cells, in both treated groups, were short when compared to normal ones at the latter age. Also, at the end of the second and third postnatal weeks, these cells appeared having shorter dendrites with missing the spicules or nodules in both treated groups as compared to normal ones (Figs. 5A$_3$–C$_3$ and 6A$_3$–C$_3$). Furthermore, these cells, in both treated groups, were shown with small size in comparison with their corresponding normal group along the duration of the experiment (Figs. 4A$_3$–C$_3$, 5A$_3$–C$_3$ and 6A$_3$–C$_3$). As well, there were some degeneration in these cells and their dendrites in hypo- and hyperthyroid groups during the examined periods (Figs.4B$_3$ and C$_3$, 5B$_3$ and C$_3$ and 6B$_3$ and C$_3$). The degeneration of neuron bodies and dendrites as well as delay in the development of dendrite arborizations are more deteriorated as a result of hyperthyroidism than hypothyroidism.

3.3.3. The internal granular layer (IGL) (Figs. 4A$_4$, A$_5$, B$_4$, B$_5$, C$_4$ and C$_5$–6A$_4$, A$_5$, B$_4$, B$_5$, C$_4$ and C$_5$)

This layer consists of two types of neurons; the Golgi and granule cells.

3.3.3.1. The Golgi cells. The Golgi cells were shown occupying the upper region of the IGL in normal and both treated groups as well as these cells appeared triangular in shape from the end of the first to third postnatal weeks (Figs. 4A$_4$–C$_4$, 5A$_4$–C$_4$ and 6A$_4$–C$_4$). Also, the dendrites of these cells were found to be branching and extending in all directions in normal and both treated groups. At the end of the first postnatal week, these cells appeared to have thick and long branches of dendrites which were produced from the different parts of the cell body in normal offspring when compared to their respective hypo- and hyperthyroid groups (4A$_4$–C$_4$). These dendrites move at different angles with the surface of the pia matter and the cell size was in the following order: normal > hyperthyroid > hypothyroid groups at the latter age. Indeed, in normal offspring, these cells had long dendrites with the presence of nodules and spicules at the end of the second and third postnatal weeks as compared to the previous age (Figs. 4A$_4$, 5A$_4$ and 6A$_4$). At the end of the second and third postnatal weeks, these cells increased in the number and size, and their dendritic branches became longer and thicker in normal offspring in relation to both treated ones (Figs. 5A$_4$–C$_4$, 6A$_4$–C$_4$). The degeneration, disorganizations and deformations were observed in both treated groups during the investigated ages in both treated groups (Figs. 4B$_4$ and C$_4$, 5B$_4$ and C$_4$ and 6B$_4$ and C$_4$).

3.3.3.2. The granule cells. The granule cells in normal and both hypo- and hyperthyroid groups were shown occupying the deep region of the IGL. These cells appeared rounded to ovoid in shape and were characterized by the presence of 1 or 2 dendrites from the end of the first to third postnatal weeks in normal and both treated groups (Figs. 4A$_5$–C$_5$, 5A$_5$–C$_5$ and 6A$_5$–C$_5$). The main dendrites branched repeatedly into secondary dendrites during the experimental period in normal offspring (Figs. 4A$_5$, 5A$_5$ and 6A$_5$). In addition, in normal offspring, these cells were found to have long dendrites as compared to both treated ones in all tested ages (Figs. 4A$_5$–C$_5$, 5A$_5$–C$_5$ and 6A$_5$–C$_5$). Also, the latter figures clearly depicted that the spicules and nodules in both hypo- and hyperthyroid groups were missing in relation to normal ones. These cells increased in the number and in their dendrites in normal offspring in comparison with both treated ones at the end of the third week (6A$_5$–C$_5$). Additionally, there were some degeneration in the dendrites, deformations and reduction in the population of these cells during the employed ages in both treated groups (Figs. 4B$_5$ and C$_5$, 5B$_5$ and C$_5$ and 6B$_5$ and C$_5$).

3.4. The cerebral neurons of offspring as shown by Golgi-Copsch stain (Figs. 7–10)

In normal and both treated groups, the layers of the cerebral cortex were characterized by the presence of the PYCs which were characterized by a pyramid-shaped perikaryon with an apical dendrite directed toward the surface of the brain and an axon leaving the base of the perikaryon to course into the white matter (Figs. 7–10). Also, the sections showed four types of nerve cells in layers III, IV, V and VI of cerebral cortex (Figs. 7–10).

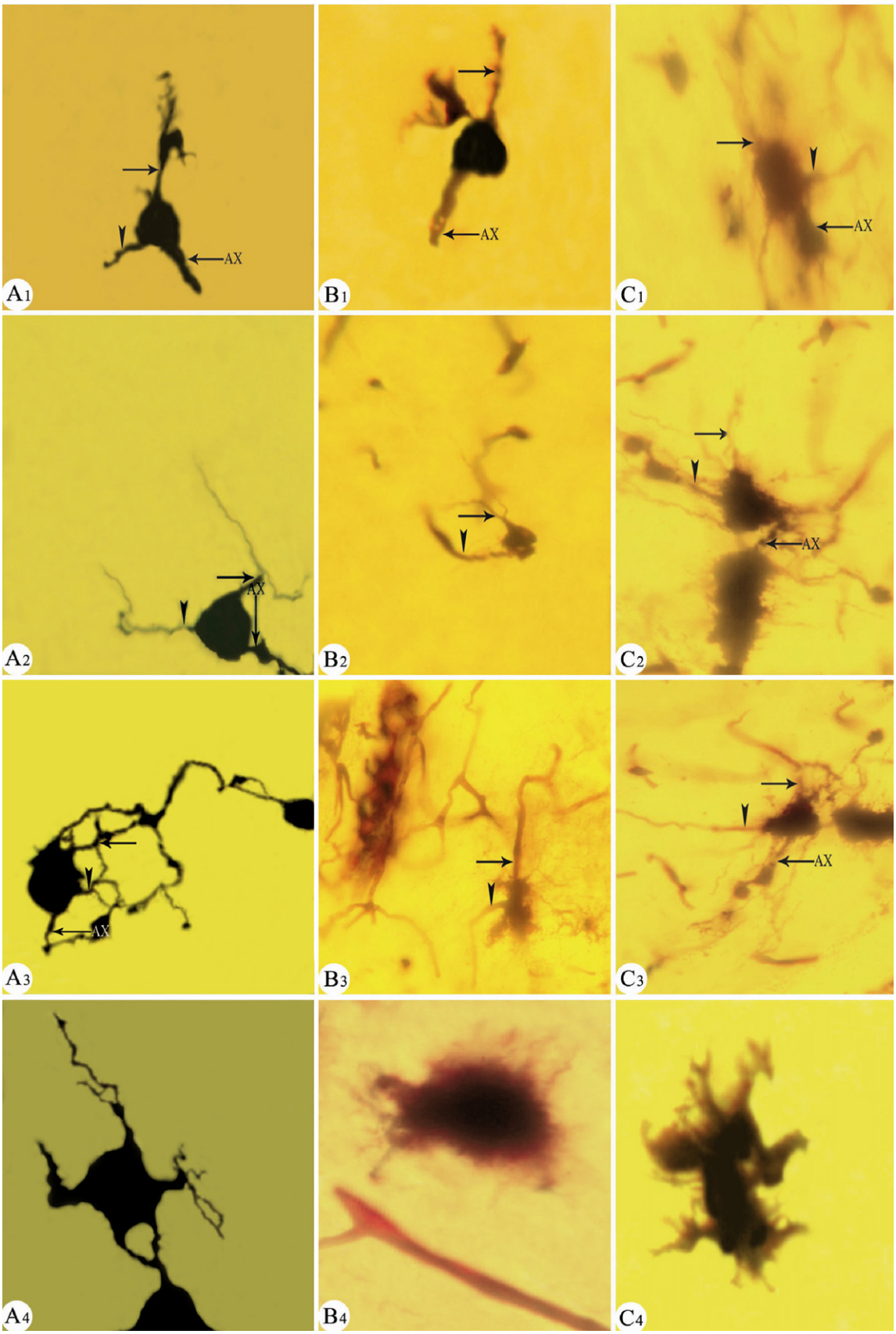

Fig. 7. Sagittal sections in the cerebral cortex showing the pyramidal and polymorphous cells in different layers at the end of the first postnatal week of normal, hypothyroid and hyperthyroid rat newborns. Pyramidal cells in layer III (A_1: Normal; B_1: Hypothyroid; C_1: Hyperthyroid). Pyramidal cells in layer IV (A_2: Normal; B_2: Hypothyroid; C_2: Hyperthyroid). Pyramidal cells in layer V (A_3: Normal; B_3: Hypothyroid; C_3: Hyperthyroid). Polymorphous cells in layer VI (A_4: Normal; B_4: Hypothyroid; C_4: Hyperthyroid). (Golgi-Copsch stain, X650) *Note*: The arrows refer to the apical dendrites. The arrows heads refer to lateral dendrites. AX refers to the axon.

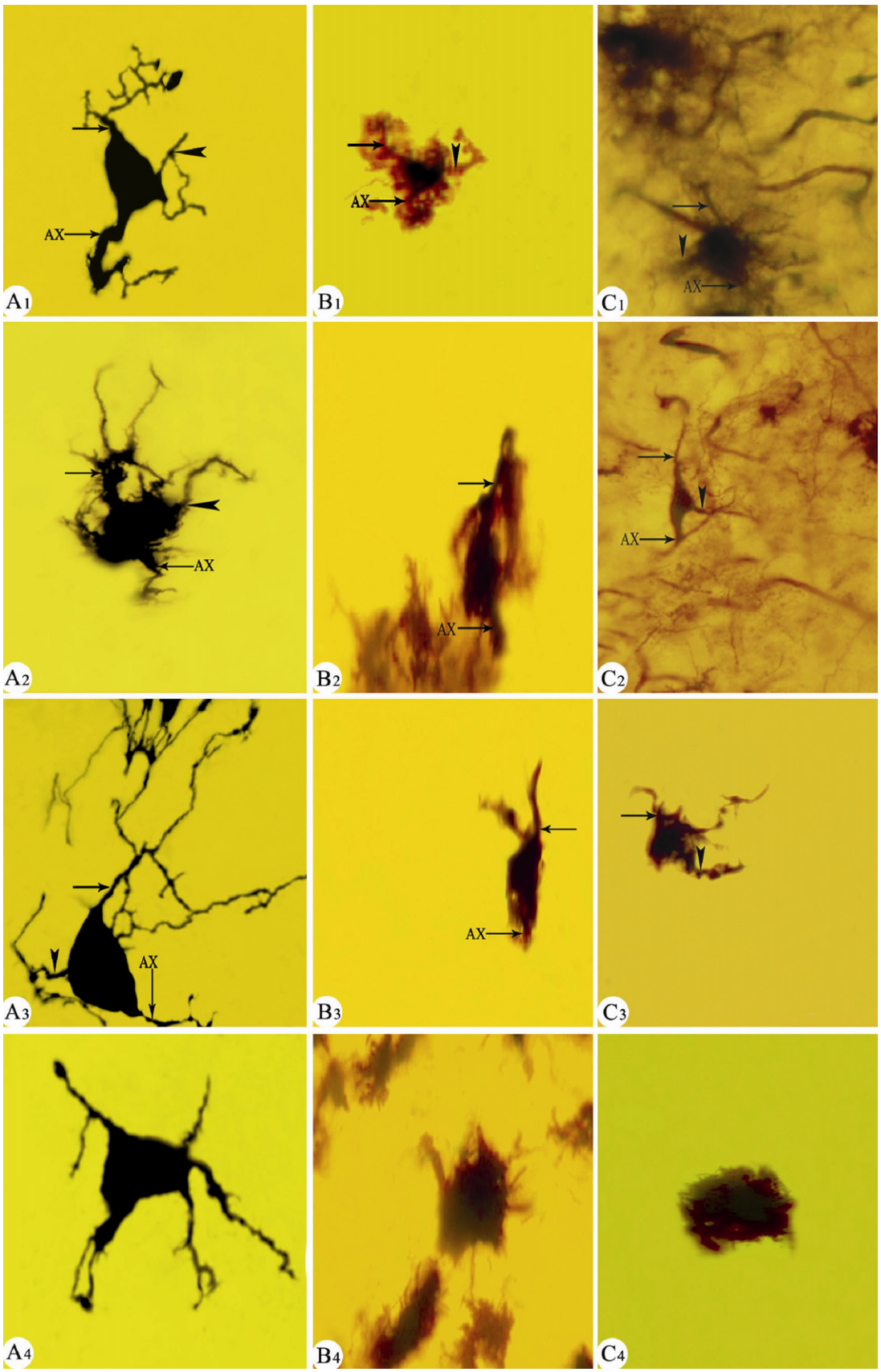

Fig. 8. Sagittal sections in the cerebral cortex showing the pyramidal and polymorphous cells in different layers at the end of the second postnatal week of normal, hypothyroid and hyperthyroid rat newborns. Pyramidal cells in layer III (A_1: Normal; B_1: Hypothyroid; C_1: Hyperthyroid). Pyramidal cells in layer IV (A_2: Normal; B_2: Hypothyroid; C_2: Hyperthyroid). Pyramidal cells in layer V (A_3: Normal; B_3: Hypothyroid; C_3: Hyperthyroid). Polymorphous cells in layer VI (A_4: Normal; B_4: Hypothyroid; C_4: Hyperthyroid). (Golgi-Copsch stain, X650). *Note*: The arrows refer to the apical dendrites. The arrows heads refer to lateral dendrites. AX refers to the axon.

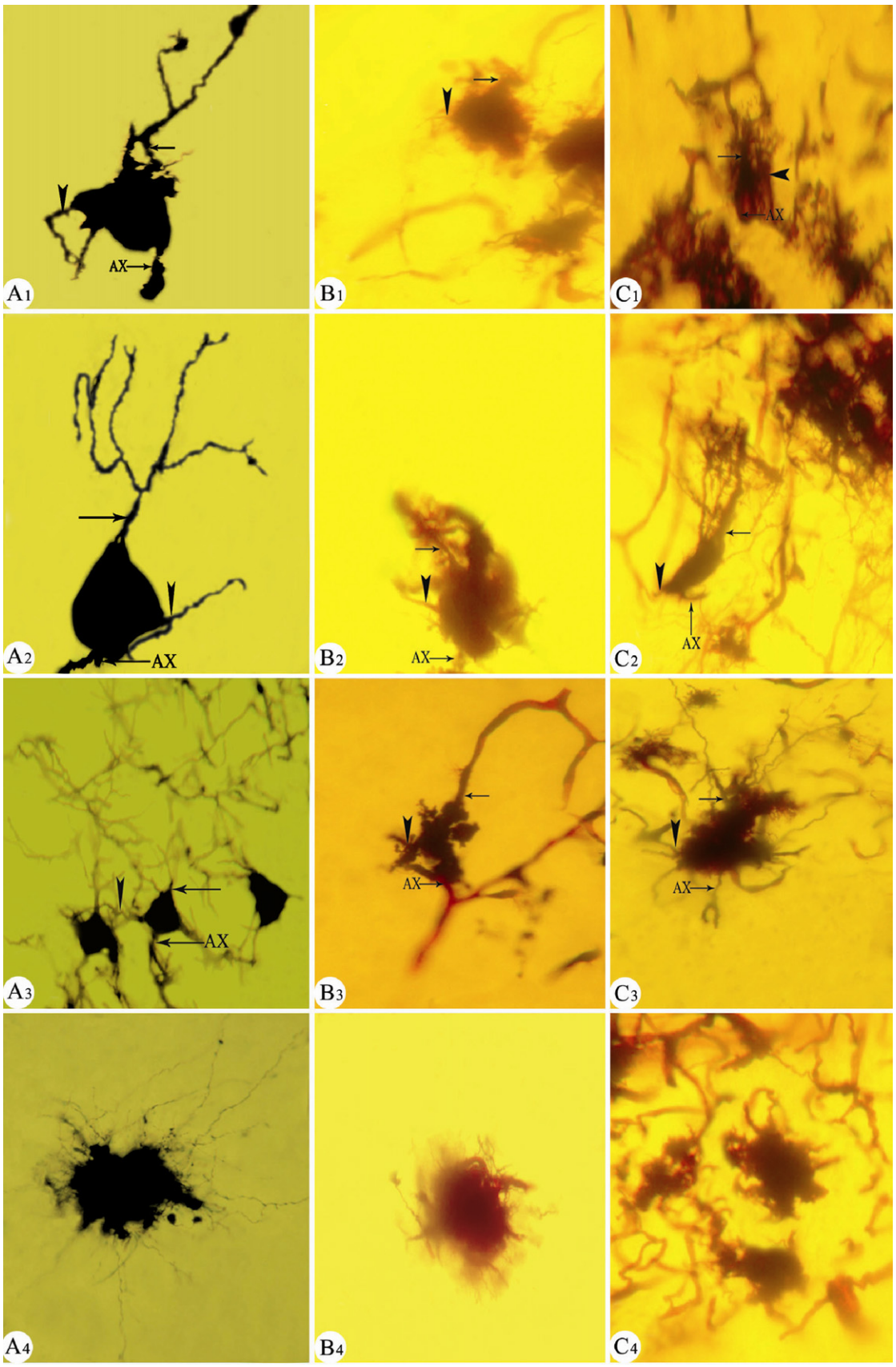

Fig. 9. Sagittal sections in the cerebral cortex showing the pyramidal and polymorphous cells in different layers at the end of the third postnatal week of normal, hypothyroid and hyperthyroid rat newborns. Pyramidal cells in layer III (A_1: Normal; B_1: Hypothyroid; C_1: Hyperthyroid). Pyramidal cells in layer IV (A_2: Normal; B_2: Hypothyroid; C_2: Hyperthyroid). Pyramidal cells in layer V (A_3: Normal; B_3: Hypothyroid; C_3: Hyperthyroid). Polymorphous cells in layer VI (A_4: Normal; B_4: Hypothyroid; C_4: Hyperthyroid). (Golgi-Copsch stain, X650). *Note*: The arrows refer to the apical dendrites. The arrows heads refer to lateral dendrites. AX refers to the axon.

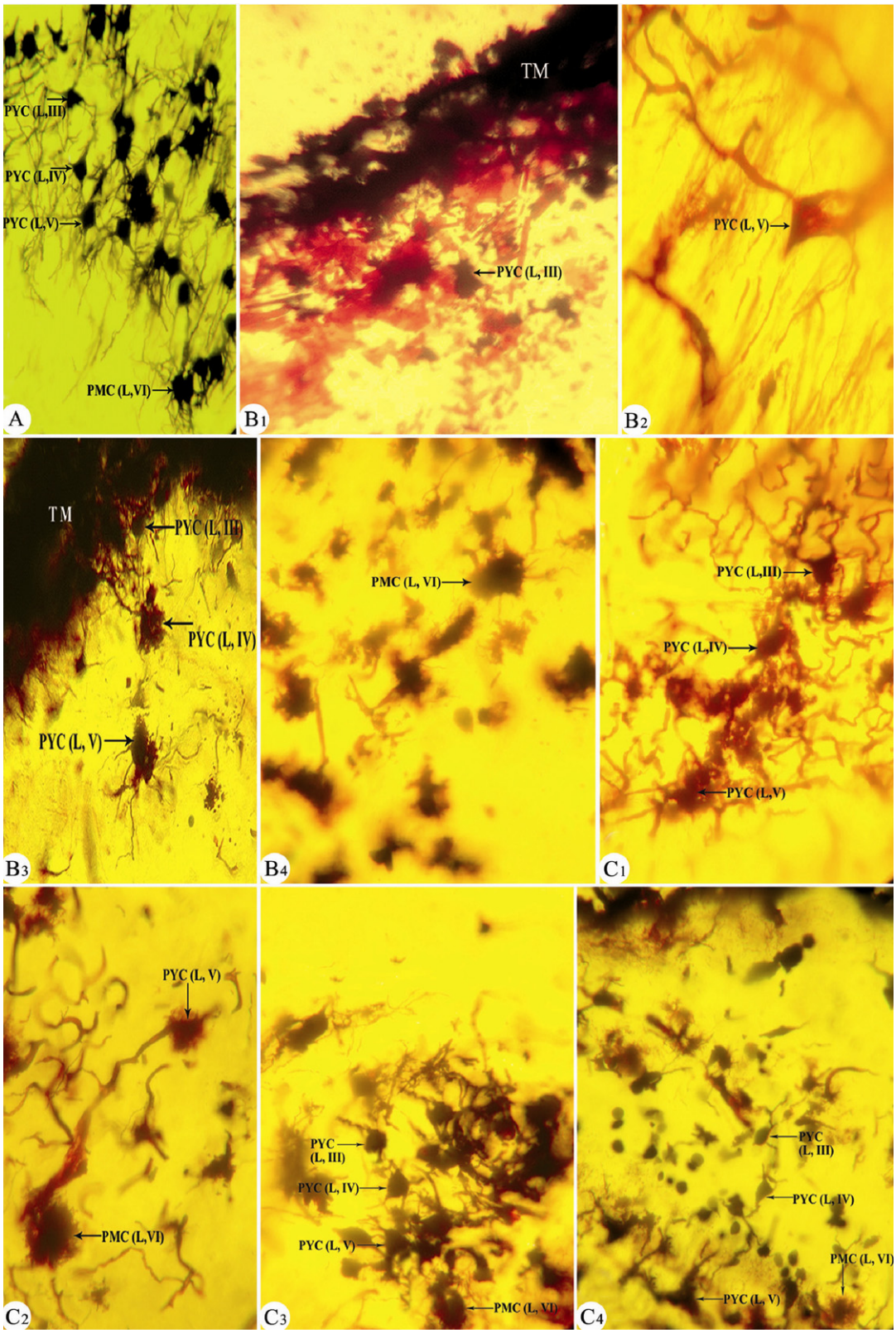

Fig. 10. General sagittal sections in the cerebral cortex showing the pyramidal (PYC) and polymorphous cells (PMC) in different layers (III, IV, V and VI) at the end of the third postnatal week of normal (A), hypothyroid (B_1–B_4) and hyperthyroid rat newborns (C_1–C_4). (Golgi-Copsch stain, X650). TM: Thickening Meninges.

In normal rat offspring, the length of the apical dendrites showed steady increase with the development in all layers of cerebral cortex. The pyramidal neurons of layer V had longer apical dendrites than those of layers III and IV in normal group and this is due to the difference in their location throughout the experimental period (Figs. 7A_1–A_3, 8A_1–C_3, 9A_1–A_3 and 10A). The spicules appeared on the apical dendrites as projections on its shaft. Also, the distribution of the spine density over the different segments of apical dendrites

showed different patterns in layers III, IV and V for all tested ages. For these reasons, the following description was based on findings from the first to third postnatal weeks in these neurons.

At the end of the first postnatal week, the neurons, in the different layers of the cerebral cortex, were clearly visualized in normal and both treated groups (Fig. 7). In normal rat offspring, the apical dendrites of PYCs in layer III appeared short and thick when compared to hyperthyroid treated ones (7A_1 and C_1) and long in relation to hypothyroid ones (7A_1 and B_1). However, there was some degeneration in PYCs in both treated groups (7B_1 and C_1). Some lateral dendrites which originate from the lateral side of the pyramidal perikaryon of layer III were noticed in normal and hyperthyroid groups and lost in hypothyroid group (7A_1–C_1). Moreover, in normal and both treated groups, the latter figures clearly showed that the axon of these cells in layer III was thick; it originates from the base of the perikaryon and moves far from the pial surface. On the other hand, in layers IV and V of normal offspring, the typical PYCs appeared with their multipolar shape (7A_2–A_3) and these cells became more complex in their dendritic fields as compared to the cells of the third layer in normal ones (7A_1–A_3). The apical dendrites of the PYCs in layers IV and V were thick, long and having some nodules and spicules in normal offspring in comparison with the respective treated groups (7A_2, A_3, B_2, B_3, C_2 and C_3). Also, the axons of PYCs in layers IV and V were short in normal offspring as compared with hyperthyroid ones (7A_2, A_3, C_2 and C_3). However, in hypothyroid offspring, PYCs was lost their axons in layers IV and V (7B_2 and B_3). The lateral dendrites of PYCs, in layers IV and V, were long in normal offspring in comparison with the corresponding hypo- or hyperthyroid ones (7A_2, A_3, B_2, B_3, C_2 and C_3). In both treated groups, layer VI was observed owning few polymorphic cells with short dendrites in respect to normal ones (7A_4–C_4). Some deformations and degeneration in the pyramidal and polymorphic cells of both treated groups were noticed at this age (7B_1–B_4 and C_1–C_4).

Moreover, at the end of the second postnatal week, the PYCs of layer III had long apical and lateral dendrites in normal offspring in comparison with both treated ones (8A_1–C_1). Furthermore, the previous figures also revealed that the axons of these cells in layer III were short in both treated groups in relation to normal ones. In normal offspring, the dendrites and axons of the PYCs increased in their lengths in layers IV and V in comparison with their respective hypo- and hyperthyroid groups (8A_2, A_3, B_2, B_3, C_2 and C_3). This development leads to clear pyramidal shape as in layer V of normal offspring (Fig. 8A_3). Also, in layers IV and V, the spicules and nodules of PYCs were noticed in normal offspring in respect to both treated groups (8A_2, A_3, B_2, B_3, C_2 and C_3). On the other hand, the lateral dendrites of PYCs of layers IV and V were lost in hypothyroid group (8B_2 and B_3) while the axon of PYCs of layer V was lost in hyperthyroid group (Fig. 8C_3). Compared with the normal offspring, in layer VI, the polymorphic cells appeared with thin and short dendrites having few spicules and nodules in hypo- and hyperthyroid groups (8A_4–C_4). Furthermore, both hypo- and hyperthyroidism caused some degenerated symptoms in the neurons and their dendrites at this stage (8B_4 and C_4).

As development proceeded to the end of the third postnatal week, the neurons increased in the number in normal rat offspring (Fig. 10A). The PYCs in different layers attained long apical and lateral dendrites in normal rat offspring; the neurons were mature with respect to the extension of their dendritic complexity (Figs. 9A_1–A_4 and 10A). At this age, the collaterals of dendrites arborizations increased in their density and complexity much more than the collaterals of the comparable layers at the previous ages in normal offspring (Figs. 7A_1–A_3, 8A_1–A_3, 9A_1–A_3 and 10A). The PYCs of layer III appeared clearly in a typical form with a pyramidal perikaryon and lateral dendrites in normal offspring (Fig. 9A_1) and these dendrites were complex in normal group than those dendrites of both treated ones in the layer III (9A_1–C_1). Also, these cells appeared with short and thick apical and lateral dendrites in both treated groups when compared to the normal ones (9A_1–C_1). In addition, the axons of PYCs cells, in layer III, were long and thick in normal offspring as compared to hyperthyroid ones (9A_1 and C_1). However, the axon of PYCs was missing in layer III of hypothyroid group (Fig. 9B_1). Moreover, as indicated in 9A_2–C_2, the apical and lateral dendrites of the PYCs, in layer IV of normal offspring, were long when compared to both treated ones. Also, the latter figures illustrated that the spicules and nodules were observed in normal and both treated groups. The PYCs of layer V, in normal offspring, reach their typical form; they had an ideal multipolar form with densely arborized apical and lateral dendrites whose collaterals were long and rich in the spicules (Fig. 9A_3). Also, 9A_3–C_3 showed that the PYCs had network tree of long dendrites in the layer V of normal offspring in respect to both treated ones. On the other hand, in hypo- and hyperthyroid groups, PYCs of layer V had thin axon and short dendrites in comparison with normal ones, however their dendrites had number of spicules in normal and both treated groups (9A_3–C_3). The polymorphic cells of layer VI had long and thin dendrites with spicules and nodules in normal offspring in comparison with their corresponding treated groups (9A_4–C_4).

4. Discussion

THs are essential both for the physiological course of pregnancy, the optimal differentiation of the embryonic tissues, normal development of body organs and integrated fetal brain development (Jiskra et al., 2007; Suchetha Kumari et al., 2011). Thus, the current study highlights the effects of maternal hypo- and hyperthyroidism on the thyroid function, neuronal development of cerebellar and cerebral cortex, and reactive oxygen species (ROS)/antioxidant defense system in different brain regions (cerebrum, cerebellum and MO) of rat offspring at the end of the first, second and third postnatal weeks.

With regards the normal maternal THs, in the present study, their serum levels [total thyroxine (TT4) and total triiodothyronine (TT3)] were lower during the pregnancy at gestation day (GD) 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 secrete THs after birth (Ahmed et al., 2010). In normal rat offspring, the current study revealed gradual increases of serum free thyroxine (FT4), free triiodothyronine (FT3), thyrotropin (TSH) and GH levels at the end of the first, second and third postnatal weeks. As suggested by Thilly et al. (1978), Momotani et al. (1986) and Porterfield and Hendrich (1992), there is a positive relationship between maternal and offspring thyroid functions. This elucidation is concomitant with the observations of the present study. Interestingly, Walker et al. (1980) elucidated that free TH concentrations in rats follow essentially the same developmental profile as do total TH concentrations. Moreover, the gradual increase of TSH herein 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 and the liberation of THs into circulation (Nosseir et al., 1991; Ahmed et al., 2008, 2010). In addition, GH is a key factor controlling postnatal growth and development (Zhou et al., 2005; Wong et al., 2006). THs are also a necessary component for the physiological growth of a young organism, stimulating the secretion of GH and insulin-like growth factor (IGF) (Wasniewska et al., 2003). In turn, these observations imply that these hormones may regulate the growth and development, in general.

The present study revealed that administration of MMI in drinking water (0.02% w/v) to adult female rats during pregnancy and weaning periods induced hypothyroidism in mothers and their offspring as indicated by decrease in serum TT4, TT3, FT4 and FT3 levels, and increase TSH. From the literature, it could be observed that the current work is in harmony with several publications using MMI-treated rats (MacNabb et al., 2000; Mookadam et al., 2004; Hasebe et al., 2008; Ahmed et al., 2010). The mechanism by which MMI exerts hypothyroidism was explained by Awad (2002) and Ahmed et al. (2008, 2010) who reported that MMI interferes with incorporation of iodine into tyrosyl residues of TG and inhibits the coupling of iodotyrosyl residues to form iodothyronine, thus inhibiting the synthesis of THs.

On the other hand, the administration of T4 to adult female rats in drinking water at 0.002% w/v beside gastric incubation of 50–200 μg/Kg b. wt. during pregnancy and weaning periods induced a marked hyperthyroidism in mothers and their offspring. This hyperthyroidism was ensured by elevated levels of serum TT3, TT4, FT3 and FT4 in these animals. Higuchi et al. (2001) and Ahmed et al. (2008, 2010) hypothesized that maternal hyperthyroidism during pregnancy and lactation leads to a hyperthyroid state in fetus and neonates. They attributed the state of hyperthyroidism in fetuses or early neonates to passive transfer of maternal T4 from a mother with hyperthyroidism or thyrotoxicosis through placenta and in mother's milk. Moreover, the decrease in serum TSH level is attributed to negative feedback effect of the excess circulating THs levels on the anterior lobe of pituitary gland. These suggestions are supported by Fisher et al. (2000) and Higuchi et al. (2005) who reported that the exposure of the fetal hypothalamic-pituitary-thyroid system to a higher-than-normal TH (T4) concentration might impair its physiologic maturation, because there is a continuous significant decrease in the TSH/fetal T4 ratio during the development.

Furthermore, while a depression in level of GH was observed in offspring of hypothyroid group, the opposite occurs in hyperthyroid group at all ages employed if compared to the levels in the age-matched normal group. Concomitant with the present results, Morreale de Escobar et al. (1993) emphasized that both plasma and pituitary GH decreased in hypothyroid fetuses from MMI-treated pregnant rats while their plasma TSH was elevated. Thus, it is worth mentioning that maternal THs deficiency may disturb the secretion of other pituitary hormones in their offspring (Wong et al., 1980; Tamasy et al., 1984). Furthermore, neonatal rat's hyperthyroidism (Varma and Crawford, 1979) results in permanent imprinting regarding growth and thyroidal development. Also, Segni and Gorman (2001) speculated that untreated childhood thyrotoxicosis causes accelerated growth.

To sum, it is observed from the above mentioned results that a transient and moderate deficiency or increase of maternal THs can have deleterious consequences on thyroid function of both mothers and their offspring. The results indicated that the thyroid functions of offspring are indirectly affected by both maternal thyroid statuses.

On the other hand, the rate of neuron development in both cerebellar and cerebral cortex was increased in its density and complexity with the age progress in the present normal rat offspring. These results are in association with the increase in the levels of neurotransmitters and activities of ATPases and deiodinase in our previous study with age progress in these brain regions (Ahmed et al., 2010). THs regulate the neuronal cytoarchitecture, neuronal growth, myelination and synaptogenesis, where their receptors are widely distributed in the CNS (Zoeller, 2004; Bernal, 2007; Ahmed et al., 2008; Leonard, 2008). This is particularly apparent in the cerebral and cerebellar cortex (Süha and Çalikoğlu, 1999). Ahmed et al. (2008) reported that the maternal THs play a critical role in CNS development and differentiation depending on the brain region and the developmental stage of their offspring.

As indicated in the current study, five types of neurons were noticed in cerebellar cortex; stellate and basket cells in molecular layer, PC in PL, and Golgi and granule cells in IGL. This result goes parallel with the work of Dalia (1998, 2002), Ahmed (2004) and Ahmed et al. (2007) in normal rat offspring. These cells were increased in number with age progress in the present normal offspring. This observation was recorded by several authors (Saleh et al., 1993; Krause and Cutts, 1994; Dalia, 2002; Ahmed, 2004; Ahmed et al., 2007). On the other hand, several authors reported that THs regulate neuronal outgrowth, proliferation and survival, and synapse formation (Oppenheimer and Schwartz, 1997; Rodriguez-Pena, 1999; Durand and Raff, 2000; Koibuchi and Chin, 2000; Thompson and Potter, 2000; Ahmed et al., 2008). In fact, Patel et al. (1987) indicated that in rats, the effect of TH on neural maturation is cell-type specific.

On the other hand, there were some degeneration and deformation in cells of molecular layer (ML) of hypo- and hyperthyroid groups during all studied ages. These observations are in accordance with those of Nicholson and Altman (1972a, b) who depicted that in rat, hypothyroidism caused retardation in cell differentiation of the ML. Moreover, Lauder (1978) recorded that the delay in cell acquisition and development results in abnormal proportions of basket and stellate cells in rat. On the other hand, hyperthyroidism (Nicholson and Altman, 1972c) in rat caused some malformations as terminal decrease in basket cells. Regarding the study herein, PCs of offspring of both hypothyroid and hyperthyroid mothers decreased in number and in their dendrites length and density as compared to normal group in all investigated ages as well as some degeneration were observed in hypo- and hyperthyroid groups. Previous results in rats showed that hypothyroidism can cause a retardation in the morphological maturation of PCs (Dussault and Ruel, 1987; Ahmed et al., 2008) as a reduction in the dendritic arborization (Nicholson and Altman, 1972a, b; Legrand, 1984), spine number (Nicholson and Altman, 1972a, b), cell size (Lauder, 1978; Vincent et al., 1983) and proliferation causing hypoplasia (Nicholson and Altman, 1972b,c; Legrand, 1984; Potter et al., 2001; Ahmed et al., 2008) during the development. Moreover, excessive doses of T4 or T3 led to an early stimulation of cell acquisition followed by a permanent deficit of cells in the cerebellum of rat (Legrand et al., 1976). It is obvious from the aforementioned results that the both treatments may retard the growth and development of PC. Concurrently, the Golgi and granule cells, in the current work, had shorter dendrites in hypo- and hyperthyroid offspring in comparison with normal ones between the first 3 weeks after birth. Also, there were some degenerated dendrites in both treated groups with the age progress. A number of investigators showed that TH affects cerebellar granule proliferation, migration, and apoptosis (Muller et al., 1995; Pasquini et al., 2000; Singh et al., 2003). The lack of TH during the critical period of neuronal migration leads to a multitude of irreversible morphological abnormalities (Porterfield and Hendrich, 1993; Bernal and Nunez, 1995) as defected in granule cell migration and its increased death (Dubuis et al., 1992; Lee et al., 2003), as well as a reduction in its number (Anderson, 2001) and parallel fiber growth (Lauder, 1978; Anderson, 2001). Furthermore, hypothyroidism causes a decrease in number and density of synaptic contacts between granule cells and PCs in rats (Legrand, 1979). Overall, a deficiency of THs in the neonatal rat has been shown to cause disorganization of the cerebellar cortex (Nicholson and Altman, 1972a, b; Nunez, 1984). On the other hand, Nicholson and Altman (1972c) reported that hyperthyroidism caused some malformations as terminal decrease in granule cells of rat. Ahmed et al. (2008) postulated that during the critical periods of the development, increase or decrease in the thyroid secretions may retard the neurogenesis and CNS

growth. According to the above results, it is worth mentioning that hypo- or hyperthyroidism may affect the growth and maturation of neurons through its effect on the vital processes of these neurons. The delayed growth and degeneration of neurons and their fibers in cerebellar cortex of hypothyroid rats are associated with the stepwise decrease in the monoamines levels and ATPase and ChE activity in our previous study (Ahmed et al., 2010). However, in hyperthyroid rats of the same study, the delayed growth and degeneration of cerebellar neurons and their fibers are associated with an increase in these variables. This controversy may be attributed to the differences in THs levels, in hypo- and hyperthyroid groups, which may directly or indirectly, affected the synthesis and expression of these neurotransmitters and enzymes.

The maturation process of pyramidal neurons of cerebral cortex involves many aspects. In normal rat offspring, the increase in the dendrites arborization with the presence of spicules is one of the maturation features. The rate of pyramidal neuron development increased herein in its density and complexity with the age progress in normal offspring. These observations are also noticed in normal rat offspring by Ahmed (2004) and Ahmed et al. (2007). The neurons of layer V in the present normal offspring precede those of layers III and IV in the maturity of all investigated ages along the duration of the experiment. This finding may show the inside outside gradient of neuron migration in white albino rat offspring. These observations are concomitant with the results of Ahmed and Gabr (2000) in normal rat offspring, and go parallel with the results of Angevine and Sidman (1961) in mice. Concurrently, THs control dendritic development and number of dendritic spines of pyramidal cells of neocortex and hippocampus (Bernal, 2003). With respect to these results, it must be mentioned that our evidences concur with the observations of Süha and Çalikoğlu (1999) and Ahmed et al. (2008) who concluded that THs play an important role in cerebral neurogenesis. The interpretation of changes in the density of spicules and nodules during the development in normal and both treated groups had to be considered in view of changes in the length of the shaft of the apical dendrites as well as its size. However, at the end of the first postnatal week, the present normal pyramidal cells of layer III had short and thick dendrites as compared to hyperthyroid group. Also, the axons of PYCs in layers IV and V were short in normal offspring as compared with hyperthyroid ones at the same period. These observations can be explained by Mussa et al. (1990) who revealed that hyperthyroidism may initially induce an acceleration of the maturation processes, including the migration and differentiation of cells, the extension of the dendritic processes and synaptogenesis. Also, Miller (1981) suggested that in the course of rat maturation, some dendrites might degenerate or fuse with adjacent ones, thus reducing the total number of primary processes.

The dendrites of the pyramidal cells of layers III, IV and V were short in the present hypo- and hyperthyroid groups in comparison with normal ones in all investigated ages except at the end of the first postnatal week in layer III of hyperthyroid group only. Further, in both treated groups, some deformations and degenerations in the pyramidal and polymorphic cells were noted throughout the experiment with lost axons or lateral dendrites of pyramidal cells in some ages. Several authors discuss these findings. Mirabella et al. (2005) reported that in infants, a lack of TH during fetal or early postnatal life is associated with specific brain damage. Deficient cellular maturation in the cerebral cortex of hypothyroid rats is characterized by (Balázs, 1973; Schwartz et al., 1997): (1) smaller neuronal cell bodies that are more tightly packed than those in euthyroid animals, (2) diminished axonal and dendritic outgrowth, elongation and branching, (3) reduced numbers of dendritic spines, (4) diminished myelination of neuronal axons (5) changes in callosally projecting neurons which may be due to the maintenance of a juvenile pattern of projections (Berbel et al., 1993, 1994, 1996, 2001; Zoeller and Rovet, 2004) and (6) alterations in dendritic morphology and structure in several cell types, including pyramidal cells in the cortex (decrease in dendritic spine number) (Schwartz, 1983). Similarly, Lavado-Autric et al. (2003) pointed out that some neurons in the neocortex of rats do not reach their normal positions in the absence of TH. These investigations led to the suggestion that TH is necessary for normal brain development in the fetus as it is during the early postnatal period (Obregon et al., 1984; Pérez-Castillo et al., 1985). In other instance, the abnormal brain development observed during hypothyroidism, in rats, may partially result from a deficiency of GH (Savard et al., 1984).

Oppenheimer and Schwartz (1997) and Ahmed et al. (2008) reported that hyperthyroidism, during the brain development, results in cytoarchitecture abnormalities and disorganization of several neuronal cells. Moreover, Sala-Roca et al. (2008) recorded that thyroxine reduces apical and basal tree of pyramidal neurons in CA3 hippocampal region. In 2008, the innovative review in the interactions between thyroid and brain development was carried out by our group has led to the conclusion that the dramatic neurological abnormalities associated with hyperthyroidism during the development testify to the importance and effect of the THs on the CNS development. Thus, worth noting in the present study is that THs dysfunctions during the critical period of development may disrupt interneuronal connections and neuronal integration.

In view of the recent studies, the current results depict important finding that the development of the investigated brain regions in rat offspring are extremely sensitive to maternal hypo- and hyperthyroidism during the pregnancy period and the first 3 weeks of lactation period. Thus, both THs defects may be responsible for the loss of neurons vital functions and decrease in their dendritic arborization. Introducing the same mode of though, 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 CNS functions (Sarkar, 2008).

ROS play an essential role in the regulation of cell proliferation, e.g. within the central and peripheral nervous system because ROS initiate and promote the establishment of neuronal patterns and subsequent neurogenesis (Verity, 1994). It was also reported that ROS play an important role in physiological processes, but – when being in excess – ROS cause oxidative damage to molecules (Karbownik and Lewinski, 2003). Antioxidant enzymes (AOEs) are an important protective mechanism against excess ROS and like many other biochemical systems, their effectiveness may vary with the stage of development and other physiological aspects of the organism (Halliwell and Gutteridge, 1999; Livingstone, 2001; Ahmed, 2004; Ahmed, 2005). Several reports have been conducted to demonstrate the modulation of the antioxidant defense system of tissues by the thyroid state of the body (Venditti et al., 1997; Pamplona et al., 1999; Tapia et al., 1999). Thus, it appears more meaningful to determine the pattern levels of the AOEs and their relationships with each other and with the oxidative markers.

The enzymatic and non-enzymatic antioxidant defense variables [glutathione reductase (GSSGR), GST, GPx, SOD, CAT, PO, PPO, vitamin C (ascorbic acid), vitamin E (α-Tocopherol), total thiol (t-SH) and glutathione (GSH)], in the present normal rat offspring, were substantially and gradually increased with the age progress from the first to third week old in all investigated regions. Previous studies reported that the overall antioxidant enzyme activities tend to increase in different brain regions with the age progress in rat (L'vova and Abaeva, 1996; Agarwal et al., 1999; Ahmed, 2004, 2005) and mice (Hussain et al., 1995). Also, the levels of non-enzymatic antioxidants were regularly increased in different

brain regions with the age progress in rat (Shivakumar et al., 1991; Ahmed, 2004, 2005) and mice (Hussain et al., 1995). Thus, based on these evidences, the increase in the activities of the tested AOEs and levels of antioxidant substrates in various brain regions (cerebrum, cerebellum and MO), in the present study, potentially reflects the enhancement in the antioxidant defense system with the age progress from PND 7 to PND 21.

Thus, based on the previous publications and results of the present study, it can be suggested that the degenerations and deformations of neurons and their dendrites in MMI-induced hypothyroidism may be due at least in part to the increase in oxidative stress and LPO as well as the deterioration in the anti-oxidant defense system.

The oxidative damage has been demonstrated, in our study, by the increased LPO and inhibition of enzymatic (GSSGR, GST, GPx, SOD, CAT, PO and PPO) and non-enzymatic (vitamin C, vitamin E, t-SH and GSH) antioxidant defense systems in most tested ages and regions of both hypo- and hyperthyroidism groups in respect to control offspring. These alterations were more pronounced at the end of the experiment. As reported by Rahaman et al. (2001), progressive hypothyroidism during the first 4 weeks of postnatal rat brain development led to a decline in the level of GSH and increase in the level of OH along with enhanced protein carbonylation and LPO; these changes are in concordance with those in the present study. The LPO is known to be involved in the damaging mechanism of several acute and chronic brain disorders of rats (Garcia et al., 2005). It was reported by Dasgupta et al. (2005) that in developing rat brain, hypothyroidism is associated with augmented oxidative stress and a decline in the level of GSH. Propylthiouracil-induced hypothyroidism can affect GST activity by depleting GSH levels that in turn affect other GSH-dependent enzymes such as GPx (Kirstein et al., 1991). Oxidative stress in MMI-induced hypothyroidism, in the present study, may initiate a cascade of events that result in cellular ionic imbalance, signal transduction and enzyme activity modifications in mammalian CNS (Janaky et al., 1999). Another possibility for the harmful effects of the antithyroid drugs like propylthiouracil and metimazole on the normal fetal development was illustrated by Awad (2002) who ascribed the high fetal susceptibility to some drugs to the decreased antioxidant enzymatic activity of the pregnant rats, which is essential for the detoxification mechanism and to the lack of development of enzymatic systems in the embryo.

Furthermore, in hyperthyroid state, several studies (Komosinska-Vessev et al., 2000; Abalovich et al., 2003; Bednarek et al., 2004a,b) have indicated an imbalance between oxidant/antioxidant status and enhanced oxidative stress in plasma and/or other tissues. Even more interesting is the elegant studies discussed by Goswami et al. (2003), Cetinkaya et al. (2005) and Guerra et al. (2005) who proposed that oxidative stress correlates with signs and symptoms of hyperthyroidism. Previously, Fernández et al. (1985) and Asayama et al. (1987) speculated that the hypermetabolic state in hyperthyroidism is associated with increases in free radicals production and lipid peroxide levels in rats. In addition, increased oxidative stress (Komosinska-Vessev et al., 2000; Abalovich et al., 2003; Reid and Wheeler, 2005) and impairment of cellular and extracellular antioxidant systems (imbalance of antioxidant/oxidant status) may play a role in the pathogenesis of Graves' disease (Komosinska-Vessev et al., 2000; Abalovich et al., 2003). Administration of L-T4 (100 μg/kg) to rabbits for 21 days caused an increase in LPO and a decrease in concentration of vitamins C and E (Kowalczyk et al., 2003; Al-Rubae'i and Al-Musawi, 2011). Mogulkoc et al. (2005) recorded that high-dose of T4 administration in hypothyroid rats increased oxidative damage in cerebrum and that this damage could not be prevented despite the increase in the antioxidant system activity. Moreover, in human, oxidative stress plays an important role in hyperthyroidism-induced tissue damage (Bednarek et al., 2004a). Also, hyperthyroidism in man is characterized by significant changes in circulating parameters related to oxidative stress as (1) increased levels of lipid peroxides (Yavuz et al., 2004; Al-Rubae'i and Al-Musawi, 2011), (2) elevated levels of H_2O_2 and lipid hydroperoxides (Bednarek et al., 2004b) and (3) reduced levels of thiols (Adali et al., 1999; Komosinska-Vessev et al., 2000), ascorbic acid (Ademoğlu et al., 1998; Seven et al., 1998), α-tocopherol (Ademoğlu et al., 1998; Bianchi et al., 1999) and GPx (Ademoğlu et al., 1998).

The depletion in GSH content as a result of hypo- and hyperthyroidism in the current study can be explained by: (1) the higher levels of free radicals that convert more reduced glutathione (GSH) to its oxidized form (GSSG) (Ou et al., 1996) and (2) decreased activity of GSSGR (Costagliola, 1991) regenerates reduced glutathione in a nicotinamide adenine dinucleotide phosphate hydrogen (NADPH)-dependent reaction. The present results support this attribution since the decrease in GSH levels in different brain regions was associated with the increase of free radicals and lipid peroxides as well as decrease in the GSSGR. In addition, Ahmed (2004) reported that the increase in the oxidative stress may be a reason for such decrease and exhaustion of antioxidant defense system, as a result of increased endogenous production of the free radicals. In general, Morozova et al. (2007) observed that GSH depletion has been implicated in neurodegenerative disorders. Furthermore, Chattopadhyay et al. (2007) speculated that reduced and oxidized glutathione were depleted in hyperthyroid rats. Previous experimental studies have commented that hyperthyroidism tended to increase the LPO products contents in all tissues (Seven et al., 1996). In conclusion, the intensification of LPO process concomitant with impairment of antioxidant potential may confirm the presence of oxidative stress in both thyroid states and disturbances in the antioxidant systems might be useful indicators of mediated oxidative stress damage. Worth noting in the present study is that low GSH content also be correlated with low GSSGR, GST and GPx activities and this association may produce increased oxidative stress propensity and depletion of the antioxidant capacities as a result of MMI-induced hypothyroidism and exogenous T4-induced hyperthyroidism. Similarly, Adali et al. (1999) reported that in human, the GSH consumption may also increase because it is used as an antioxidant against oxidant stress in hyperthyroidism.

Hence, the decreased levels of antioxidants should create an imbalance between pro-oxidant and anti-oxidant systems in both hypo- and hyperthyroid groups, thus stimulating the harmful effects in all tested regions through a deleterious release of the free radicals. Thus, our study may provide an evidence for the adverse correlation between the changes of LPO and the alteration in antioxidant defense system in all examined regions. The compensatory increase in some enzymatic antioxidant system (GSSGR, SOD, CAT, PPO and PO), in offspring of hypo- or hyperthyroid groups, in the present study, during the first two weeks in some studied regions, may be a trial from the antioxidant defense system to acclimatize the new condition in which excess free radicals and lipid peroxides are produced. However, the continuation of exacerbated levels of lipid peroxides and ROS for longer periods (third week) may lead to exhaustion and reduction in the levels of some AOEs. This assumption was supported well with various publications. Ademoğlu et al. (2006) emphasized that the increased activity of the enzymes of the antioxidant defense system (GPx and SOD) and the increased t-SH concentrations in thyroid tissue indicate the reactive response to increased FR generation as well as this suggests that the total peroxidation capacity is influenced by the thyroid state of the body.

From the pre-said studies, it is also worth mentioning that the thyroid-antioxidant system interactions could protect the cells and tissues, in general, during the normal development from the

harmful effect of ROS. Altogether, the results are suggestive of the imbalance of generation and scavenging of free radicals and ROS, in both thyroid states, may play an important role in determining a partial retardation in neuron development. These investigations bear certain resemblance to several publications, which revealed that the oxidative stress and damage play a role in the pathogenesis of a number of diseases associated with neurodegeneration and accelerated cell death by means of either apoptosis or necrosis (Bednarek et al., 2004a; Ferriero, 2004; Ahmed et al., 2006; Fernández et al., 2006; Kolosova et al., 2006).

In conclusion, THs, in normal state, play a crucial role in the development and physiological functioning of the CNS. The MMI-induced hypothyroidism and exogenous T4-induced hyperthyroidism may cause a number of injurious anomalies in the development including degeneration, damage, disorganization and deformation of neurons and dendrites. These deteriorations may be attributed, at least in part, to the increased oxidative stress and impaired anti-oxidant defense system in both thyroid states.

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