

# Thyroid Hormones and Motoneurons Development

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## 1. Introduction

It is well known that postnatal maturation of the central nervous system is critically dependent on thyroid hormone levels (Thompson & Potter, 2000) and this might influence the neuromuscular system (Barakat-Walter et al., 2000). Previous neuroanatomical and biochemical investigations demonstrated that development of skeletal muscles including the masseter is affected by both neuronal and thyroid hormonal effects (Adams et al., 1999; d'Albis et al., 1989, 1990; Butler-Browne et al. 1984; Gambke et al., 1983; Rubinstein et al., 1988). Under normal condition the phenotypic properties of motoneurons and muscle fibers in the neuromuscular unit are matched (Coprav & Kernell, 2000; Hughes & Salinas, 1999; Akihiko Ishihara, Kawano, Okiura, Morimatsu, & Ohira, 2005).

A pronounced shift in oromotor behavior occurs with the transition from sucking to chewing in humans and other mammals (Green et al., 1997; Saito, Ohnuki, Yamane, & Saeki, 2002). It has been reported that the transition from neonatal to adult fast MHC is however dependent on thyroid hormone (Soukup & Jirmanová, 2000). In the rat there is a significant rise to peak T4 serum levels at 15 days followed by a slight decline to mature values (Gambke et al., 1983). The diameter of muscle fibers enlarges progressively from slow to fast type in order to adapt to the rapid functional changes from weaning to chewing motion (Miyata et al., 1996).

During development, hypothyroidism results in an inhibition in the expression of adult fast MHC isoforms and a persistence of the slow isoforms in the masseter muscle (Agbulut et al., 2003; Butler-Browne et al., 1987; Pette & Staron, 2000) which is also associated with a decrease in fiber diameter of the masseter muscle (Sugasawa & Mori, 1998). The increase in the circulating levels of thyroid hormone in suckling rats is involved in development of the masseter (Maeda et al., 1981a, 1981b). This effect is explained first as a result of an orthograde mechanism through the trophic factors secreted by different motoneuron types at the neuromuscular junction. The second explanation invokes a retrograde mechanism, so that, once muscle fibers are differentiated into slow or fast types they may modify properties of motoneurons via retrograde transport of substances (Barakat-Walter & Riederer, 1996; Munson et al., 1997). Based on these hypotheses, Sickles et al. (Sickles et al., 1987) and Bakels et al. (Bakels et al., 1998) reported considerable alteration in the adult rats' soleus motoneurons morphology due to hyper- and hypo-thyroidism respectively. In regard to the

masseter muscle, neuroanatomical evidence related to the mechanisms of shifting from sucking to biting was first reported by Kubota et al. in mice (Kubota et al., 1988). Upon their observation the differentiation of the trigeminal motoneurons related to biting is rapidly accelerated after birth. Miyata et al. have reported morphometric alteration of superficial masseter motoneurons from sucking to chewing in normal rats in a way that the diameter of the largest motoneurons increases rapidly from 5 to 21 postnatal days (Miyata et al., 1996).

Calcitonin-gene related peptide (CGRP), a co-transmitter, along with acetylcholine in the neuromuscular system is released at motor end plates, where the muscle cells demonstrate binding sites for CGRP (Popper & P. E. Micevych, 1989; Terrado et al., 1997). In fact, CGRP is synthesized in the motoneuron cell bodies, transported down to the motor terminals, stored in dense-core vesicles and released upon nerve stimulation (Buffelli et al., 2001). This neuropeptide exerts a variety of effects on skeletal muscle such as spontaneous acetylcholine release from motor nerve terminals, enhancement of neurally evoked muscle contraction and regulation of the rate of the acetylcholine receptor (AChR) at the neuromuscular junction (Kimura, 1998; van Rossum, Hanisch, & Quirion, 1997). CGRP functions are mediated by cell membrane receptors that belong to the family of G-protein-coupled receptors (van Rossum et al., 1997). CGRP may thus serve as an anterograde trophic agent released by motoneurons that contributes to the maintenance of a high density of neuromuscular junctional AChRs (H L Fernandez, Chen, I Nadelhaft, & Durr, 2003; Roa & Changeux, 1991). Indeed, motoneuronal CGRP acts as a physiological transducer through its complex receptors in muscle motor endplates (Hugo L. Fernandez, Ross, & Irving Nadelhaft, 1999). It has been proposed that the levels of CGRP present in individual motoneurons are related to the type of muscle unit that is innervated by the respective motoneuron (Popper & P. E. Micevych, 1989). It has also been claimed that there is a relationship between the type of myosin composition of different rat muscles and the CGRP mRNA expression in conveying motoneurons (Blanco, Popper, & P. Micevych, 1997). Hypothyroid muscles show a nearly 50% reduction in AChRs density when compared to the control muscles (Kragie & Smiehorowski, 1993), therefore during weaning, when feeding behavior needs to transform from suckling to chewing (Saito et al., 2002), prenatal slow myosin persists, preventing faster muscular contraction due to severe decrease in the density of neuro-muscular junction AChRs (Miyata et al., 1996). According to the available target, motoneuronal CGRP levels alter in relation to the type of muscle fibers (Blanco et al., 1997; Popper & P. E. Micevych, 1989).

A review of literature regarding to motoneurons development shows that detailed morphometric data on the developing masseter innervation has been neglected in prenatal hypothyroid rats. Thus in this chapter the morphological features of the developing masseter motoneurons labeled by injection of HRP into the superficial masseter muscle were analyzed in normal and congenital hypothyroid rat's offspring. HRP retrograde reaction product is observed as dark blue intracellular granules varying in quantity from motoneuron to motoneuron even in the same trigeminal motor nucleus (Kawagishi et al., 1992). As hypothyroidism reduces neuronal process growth, synaptogenesis, axonal transport velocity (Biesiada et al., 1996; Stein et al., 1991) and neurotransmitter synthesis (Barakat-Walter et al., 2000; Behzadi & Ganji, 2005), it was of especial interest to investigate the alteration in the morphological characteristics of masseter motoneurons as well as HRP uptake and transport from the neuromuscular junction. In this regard the labeling quality of HRP backfilled masseteric motoneurons along with their size distribution profile under

developmental hypothyroidism were evaluated. Furthermore the oro-facial motoneuronal CGRP immunoreactive responses under the congenital thyroid hypofunction were examined and also using the Golgi staining method, the morphology of the masseteric motoneurons including their dendritic arborization pattern in normal and hypothyroid weaned rat pups was studied. These studies may lead to better understanding of the ontogenic changes in mastication.

## **2. Materials and methods**

### **2.1 Animals**

Timed pregnant Sprague-Dawley rats (Pasteur's Institute, Tehran, Iran) were housed individually in plastic cages with free access to food and water. The animal room was maintained at constant 22-24° C temperature under a 12 hour light/ 12 hour dark cycle. The studies were performed according to the guidelines for laboratory animal use and care set forth by the research council at Shahid Beheshti University of Medical Sciences (Tehran-Iran). Neonatal hypothyroidism was induced by adding 50mg/liter PTU (Sigma) to the drinking water of pregnant dams beginning at gestational day 16 to postnatal day 23. It should be noted that this concentration represents the same amount of PTU which is received by the pups during suckling period (Blake & Henning, 1985). Control dams received tap water. Usually litters were culled to 8 pups on postnatal day 1 for each dam.

### **2.2 Intramasseter HRP injection**

On the 1st, 5th, 13th and 21st days after birth several male pups in each age group were anesthetized by i.p. injection of Ketamine (100mg/Kg) and Xylazine (5mg/Kg). A small incision was made in the chick skin to expose the surface of the superficial masseter muscle. Then 1-5µlit of 40% HRP (type VI- Sigma) dissolved in sterile saline was injected slowly into the 2-5 loci above as well as under the parotid duct in the left masseter using a Hamilton syringe as demonstrated by Kawagishi et al. (Kawagishi et al., 1992). After each injection the needle was left in situ for 1 min to avoid backflow of the injected HRP, following which the needle was removed, the injection sites were cleaned with sterile saline, and the opening was sutured.

#### **2.2.1 Histochemical procedure**

After 24-48h of survival time, the pups were deeply anesthetized and perfused transcardially with 20-50 ml saline (37°C) followed by 50-100 ml of fixative ( 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4, 4°C). Following perfusion and fixation the lower brainstems were removed and post fixed for 24h. The blocks of tissue were cut serially into 50µm thick coronal sections using a Vibratome. Then the sections were processed for HRP reaction using TMB method (Mesulam, 1982) and counterstained with 0.1% neutral red.

#### **2.2.2 Microscopic study**

In each experimental group, three pups with the most reliable labeling in their trigeminal motor nucleus (Mo5) were chosen for microscopic study. From rostral to caudal part of Mo5,

eight cross sections were selected per animal for each age group of normal and hypothyroid pups. The HRP labeled motoneurons showing a nucleolus or with visible primary dendrites were counted and upon their HRP labeling profile they were semi-quantitatively divided into strong (S) and weak (W) intensities. The cell body area of 500 HRP labeled neurons with both intensities were measured through the cross sections using a computer based image analysis system (Olympus BX60, DP12, Olysia soft imaging system, Japan). To measure the soma areas images of labeled cells were displayed on a monitor and their cell bodies perimeters in continuous with soma-dendritic transitional regions were outlined. Photomicrographs were arranged using CorelDRAW12.

### 2.2.3 Statistics

Differences between normal and hypothyroid groups were analyzed with two tailed student's *t*-test. Two- way analysis of variance (ANOVA) was employed to assess the variation of soma size in relation to labeling intensity of masseteric motoneurons in different groups. The level of significance was set at  $P < 0.05$ . Values are means  $\pm$  SEM

### 2.3 CGRP immunohistochemistry

At the onset of weaning period (post natal day 23), 12 deeply anaesthetized (100 mg/kg ketamine and 5 mg/kg xylazine) male pups (6 hypothyroid and 6 controls) underwent transcardial perfusion with saline followed by 4% paraformaldehyde, 1.33% picric acid and 0.1% glutaraldehyde in phosphate buffer, pH 7.4. The brainstems of all 12 rats were cut serially into 50 micron thick coronal sections with a Vibratome. Tissue sections were collected in phosphate buffered saline (PBS) containing 0.3% Triton X-100 (PBST) and 0.3% hydrogen peroxide. The sections were rinsed with bovine serum albumin (0.1% in PBST, for 1 h) and then they were incubated in a rabbit polyclonal CGRP antibody solution (Sigma, USA, 1:2500 dilution) at 4 °C for 72 h. Thereafter, the sections were washed in PBST and incubated in biotinylated goat anti-rabbit IgG (Vector Laboratories, 1:2000 dilution) at 4 °C overnight with stirring. After two further rinses, the sections were placed in a 1:1000 dilution of avidin-biotin complex (Vector Laboratories) for 2 h. The immunohistochemical reaction product was revealed with 0.05% 3, 3'-diaminobenzidine (DAB, Sigma) and 0.5% nickel in Tris-HCl buffer, pH 7.6, in the presence of 0.005% hydrogen peroxide. Finally, tissue sections were washed in Tris-HCl buffer, mounted on gelatinized slides and counterstained with neutral red before coverslipping

#### 2.3.1 Microscopic study

Eight sections containing main and accessory trigeminal (Mo5 and Mo5-AC) and facial (Mo7) motor nuclei were selected per animal. A semiquantitative CGRP-like intensity of these motoneurons was unilaterally evaluated as strong, moderate, weak and negative staining. The mean soma diameter of the motoneurons showing a nucleus were obtained by taking the average of two diameters, measured at the maximal and minimal axes of soma (Honma et al. 2002; Ishihara et al. 1988), using a computer- based image analysis system (Olympus BX60, DP12, Olysia soft imaging system, Japan). Photomicrographs were arranged using CorelDRAW12. Statistical significance was analyzed by Student's *t*-test. The level of significance was set at  $P < 0.05$ .

## 2.4 Golgi staining method

Time pregnant female Wistar rats weighing 180 g were randomly divided into control and PTU-treated groups. PTU-treated group received 50ppm propylthiouracil (PTU) in their drinking water from 16<sup>th</sup> day of pregnancy, continued to 22<sup>nd</sup> day post-partum. Control group received tap water. After transcardial perfusion, brain stems of 6 male 23 day old pups in each experimental group were processed for Golgi-Hortega staining method. Using rotary microtome brain stem paraffin embedded blocks were cut to 70 micron slices. Mo5 tissue sections were selected for photomicrography and morphological analysis. Using Image Analysis Starter software (Olympus, Japan) the cross section area of selected motoneurons in both experimental groups were measured and their primary and secondary dendrites were counted. Dendritic tree arborization pattern was analyzed with altered Sholl's concentric circles method (Ristanović, et al. 2006) . Statistical analysis including Student's t-test and ANOVA tests was done using SPSS software.

## 3. Results

### 3.1 Experimental hypothyroidism

For induction of prenatal hypothyroidism, at first, a range of 0.050 and 0.075 % of PTU was tested, but the survival of the pups dropped sharply beyond the second postnatal week and and finally the 0.005% concentration of this drug was used which induced a mild hypothyroidism and allowed us to have hypothyroid pups with a moderate rate of mortality until the time of weaning. In accordance with previous observations (Blake & Henning, 1985; Sawin, Brodish, Carter, Stanton, & Lau, 1998), PTU treated pups displayed the skeletal and morphological deformities characteristic of hypothyroidism including blunt snouts, unfolded ears and rounded bodies compared to normal pups, eye opening was delayed for 2 days. In this study, PTU-treated pups were weighed at different times from birth to 23 days after birth (Fig 1). At the time of weaning hypothyroid pups weighed 50% under normal weight.

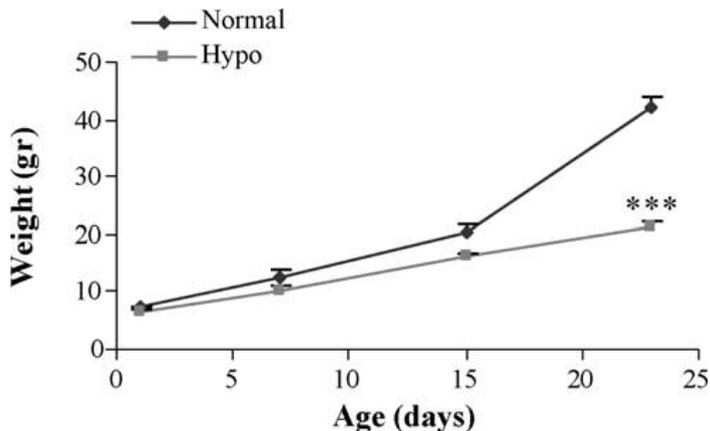


Fig. 1. Body weight profile (mean  $\pm$  S.E.M.) of hypothyroid offspring was significantly reduced compared to the controls by postnatal day 15 up to 23 ( $P < 0.001$ ).

### 3.2 Masseter HRP labeled motoneurons

In accordance to Mizuno et al., the masseteric labeled motoneurons were located in the dorsolateral part of the trigeminal motor nucleus (Mizuno et al. 1975). No contralateral neuronal labeling was observed. Unlabeled motoneurons were excluded from the study. The labeled motoneurons of normal and hypothyroid pups at days 1, 7, 15 and 23 are shown in Fig 2. The number of labeled motoneurons in control pups was not significantly different from those found in their hypothyroid homologues (table 1). In addition, the total number of strongly labeled motoneurons (S) was higher than weakly labeled (W) ones in both normal and hypothyroid pups during postnatal development. Indeed, the most obvious morphological changes in hypothyroid masseter motoneurons could be detected from day 15 to 23, in that the hypothyroid masseteric motoneurons showed less primary dendrites with shorter processes and slightly more weakly HRP labeled soma compared to normal pups (fig. 3).

Pups	day 1	day 7	day 15	day 23
Normal	190±18.8	170±13.9	195±19.1	174±17.0
Hypothyroid	182±24.0	182±22.7	199±13.5	176± 7.8

Table 1.

The correlative results between soma size and HRP labeling intensity of 500 measured motoneurons in each group were as follows:

#### Day 1

At day 1 after birth, a similar number of HRP-positive neurons was found in hypothyroid and in normal pups. The soma area of the labeled motoneurons ranged between 80-400 $\mu\text{m}^2$ . Among them, the number of smaller motoneurons (soma area < 200 $\mu\text{m}^2$ ) was about 2/3 of all the labeled cells (Fig 4A). In addition, about 60% of them were strongly labeled (table 2).

#### Day 7

One week after birth the masseter motoneurons grew rapidly and about 4/5 of total labeled cells reached a soma area of 200 up to 500 $\mu\text{m}^2$  and the ratio of S /W neurons was about 2/1 in both normal and hypothyroid pups (tables 2,3).

#### Day 15

The medium size motoneurons appeared at the 15th postnatal day with a significantly lower value in hypothyroid pups ( $P < 0.001$ , table 2). In contrast their small sized weakly labeled motoneurons (<500 $\mu\text{m}^2$ ) were higher than normal ( $P < 0.01$ , table 2,3). Regardless of the labeling intensity a higher frequency of smaller motoneurons (<300 $\mu\text{m}^2$ ) and a lower frequency of larger motoneurons were observed in hypothyroid pups when compared to normal pups (fig. 5A). Both the intense and weakly hypothyroid labeled neurons displayed quite shorter processes in comparison to normal animals (Figs 2, 3).

#### Day 23

The most pronounced changes in soma area and labeling intensity were observed at the time of weaning. Small motoneurons in normal pups comprised less than 20% of all labeled

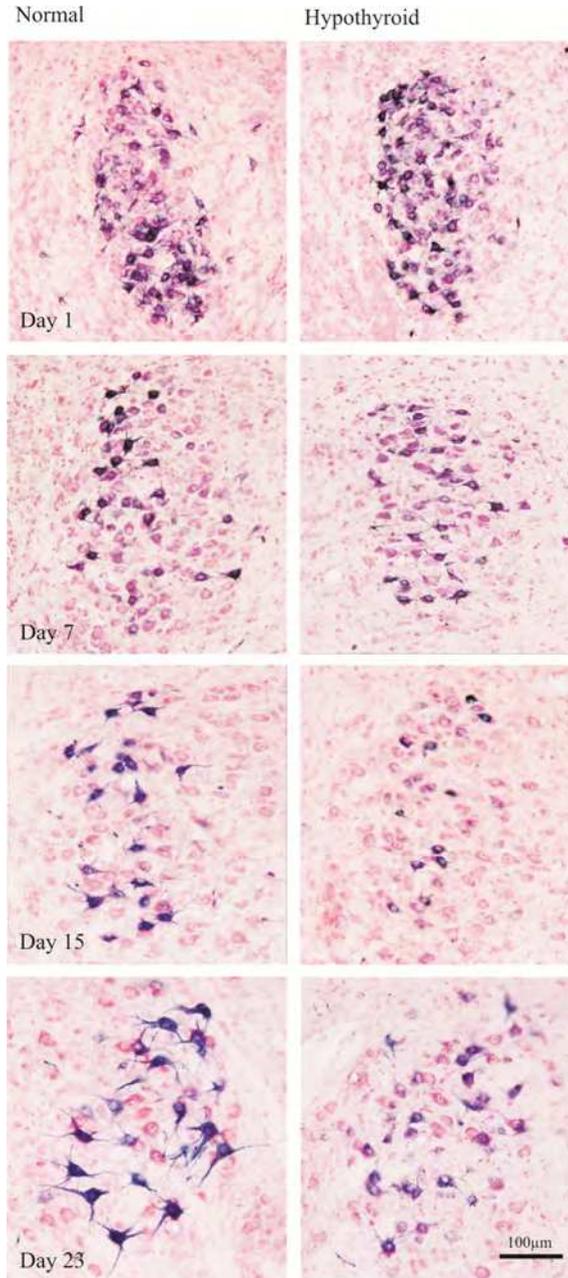


Fig. 2. Photomicrographs showing superficial masseter HRP labeled motoneurons from day 1 to day 23 in normal and hypothyroid trigeminal motor nucleus. At day 23 initial parts of dendrites of normal labeled motoneurons exhibit a Golgi-like labeling appearance with visibly longer extension than those of their hypothyroid homologues.

motoneurons, whereas hypothyroid Mo5 contained 2 fold more of small motoneurons ( $P < 0.001$ ). The medium size motoneurons had almost an equal quantitative pattern (~ 45% and 50%) in both normal and hypothyroid pups respectively. While the number of large motoneurons reached to 40% of total number of labeled motoneurons in normal pups, the hypothyroid masseteric motor pool contained 15% of large cells ( $P < 0.001$ ), (See fig. 5B in details). The pattern of labeling intensity showed larger and strongly HRP labeled motoneurons in normal pups versus smaller and strong intensity of HRP labeled motoneurons in hypothyroid pups. In normal animals, the Golgi-like labeled motoneuron pool had numerous dendritic processes that extended in both ventral and transverse directions. However in hypothyroid motoneurons the primary dendritic processes were shortened remarkably in all any directions (Fig. 3, B, b).

Peak frequency distributions of labeled motoneurons with different soma size revealed a trimodal pattern of masseter muscle innervation at the time of weaning in both groups: Small size motoneurons with soma area  $< 500 \mu\text{m}^2$ , medium size motoneurons  $500\text{-}700 \mu\text{m}^2$  and large motoneurons  $> 700 \mu\text{m}^2$  are presented in table 2.

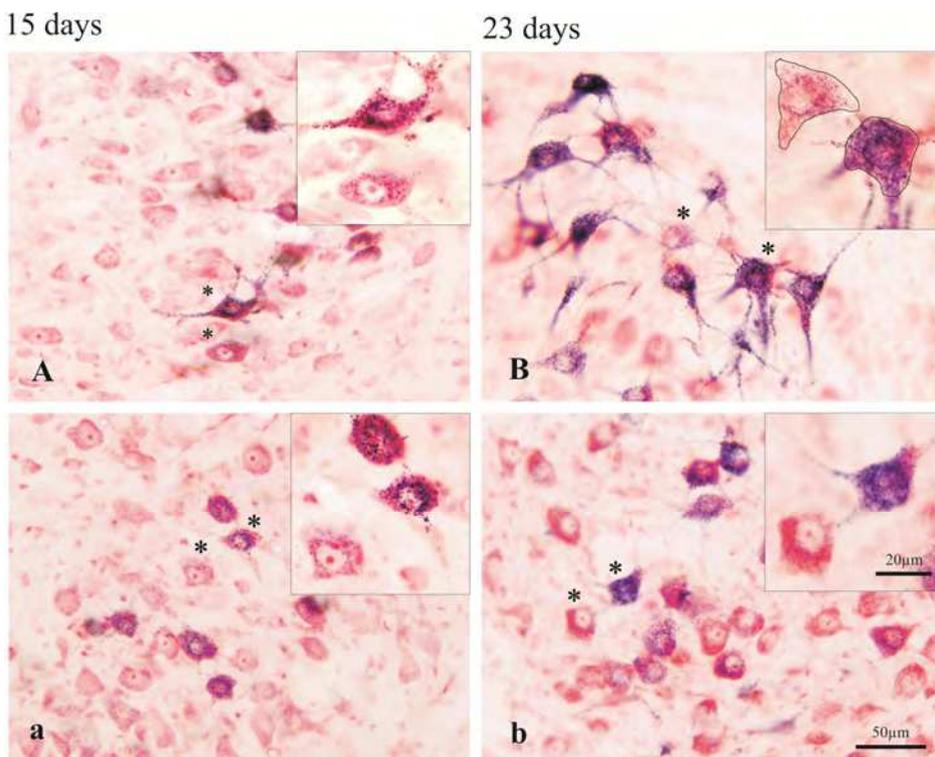


Fig. 3. Masseteric motoneurons labeled with HRP at 15 and 23 postnatal days in normal (A, B) and hypothyroid (a, b) pups. Insets illustrate high magnification of strong and weakly labeled motoneurons (asterisks). Examples of outlined motoneurons are shown in B (inset). Note that hypothyroid motoneurons possess remarkably shorter dendritic processes compared to those of normal pups.

	Strongly labeled cells						Weakly labeled cells					
	small		medium		large		small		medium		large	
	N	H	N	H	N	H	N	H	N	H	N	H
Day 1	96± 3.9	96± 4.8	0	0	0	0	71± 8.4	70± 6.1	0	0	0	0
Day 7	117± 8.0	106± 5.7	0	0	0	0	51± 2.7	61± 7.0	0	0	0	0
Day 15	97± 3.1	92± 2.1	15± 0.3	6± 0.9***	0	0	43± 1.5	62± 3.6**	13± 0.6	4± 0.7***	0	0
Day 23	17± 0.9	40± 3.0**	44± 3.8	50± 4.2	52± 3.2	13± 1.5***	15± 1.1	31± 2.1**	24± 1.9	26± 3.3	15± 2.3	6± 0.6***

N; normal, H; hypothyroid. \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

Table 2.

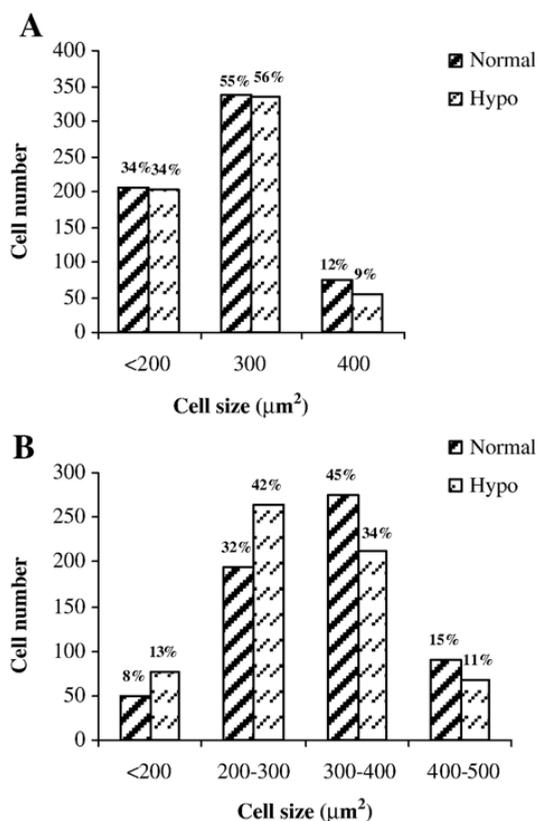


Fig. 4. Frequency distributions in soma area of labeled motoneurons innervating superficial masseter muscle in both normal and hypothyroid pups at 1 day of age (A) and 7 days of age (B). There was no significant difference between normal and hypothyroid pups in both age groups, although at 7 days of age in hypothyroid pups the number of smaller cells (up to 300  $\mu\text{m}^2$ ) was more and that of larger cells (300–500  $\mu\text{m}^2$ ) was less than the normal pups.

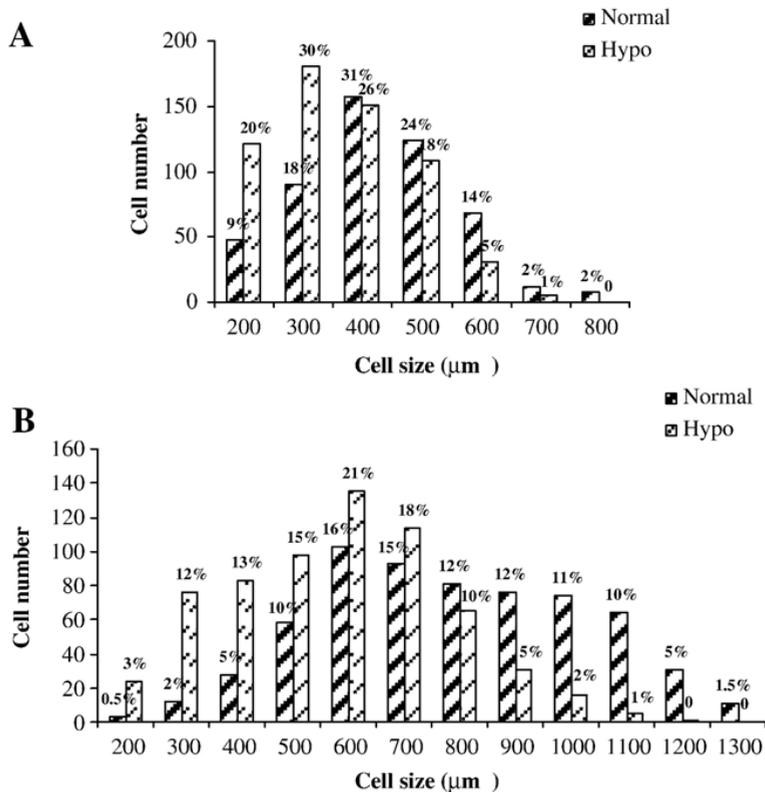


Fig. 5. Frequency distributions in soma area in both normal and hypothyroid pups at 15 days of age (A) and 23 days of age (B). At day 23, neurons in both control and hypothyroid pups were composed of three populations with lower quantity of small, large size and higher quantity of medium-sized motoneurons in peaks. Note that in normal pups there are significantly more larger and less smaller motoneurons than in hypothyroid pups.

### 3.3 CGRP Histochemistry

To analyze the effect of prenatal thyroid hypofunction on the CGRP immunoreactive intensity, distribution of CGRP containing motoneurons was quantified through Mo5, Mo5-AC and Mo7 nuclei (Fig. 6). CGRP immunoreactivity is extensively and differentially expressed in oro-facial motoneurons somata and primary processes.

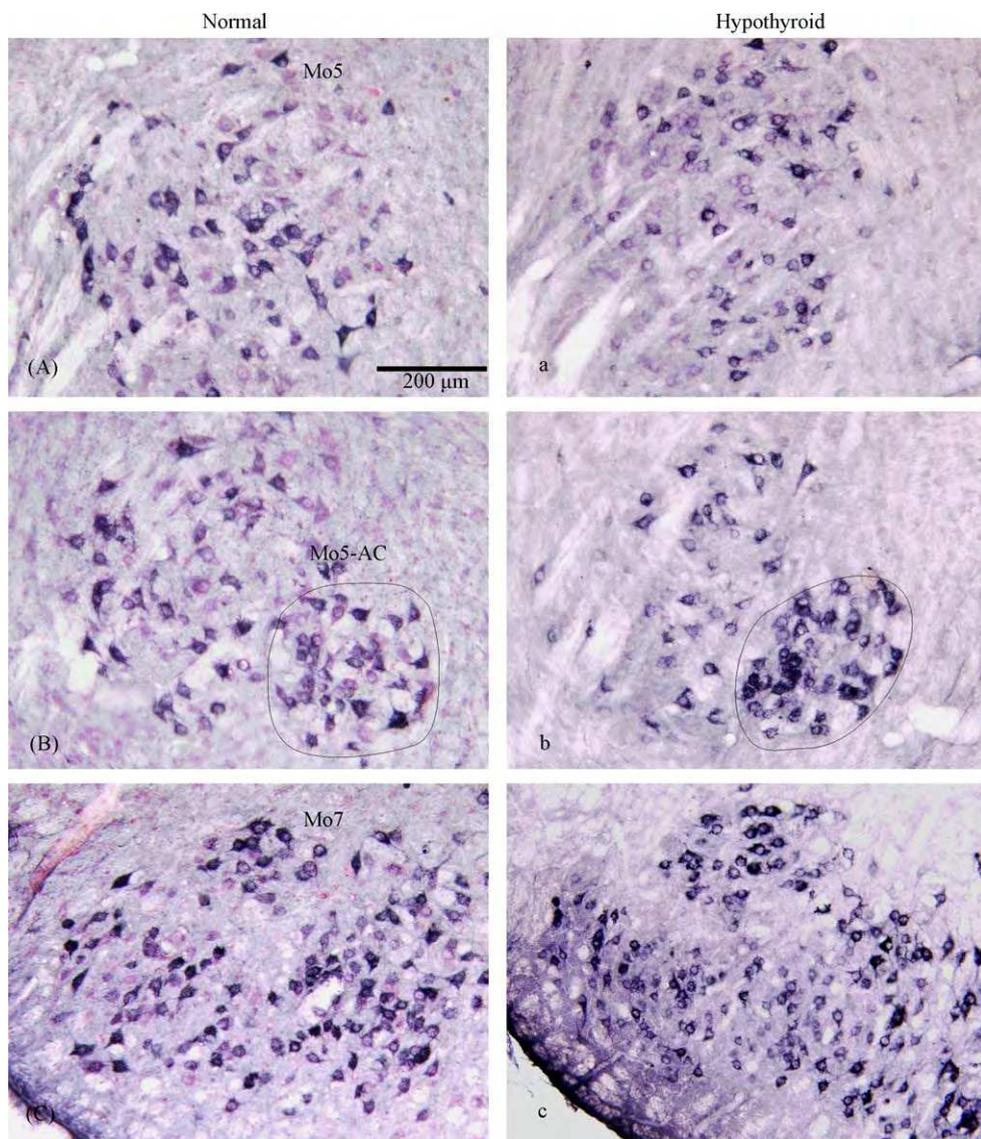


Fig. 6. Low-power photomicrographs of frontal sections through brainstem showing the distribution of CGRP immunoreactive motoneurons through the trigeminal (Mo5), trigeminal accessory (Mo5-AC, outlined area) and facial (Mo7) motor nuclei in the normal (A-C) and in the hypothyroid (a-c) weaned pups.

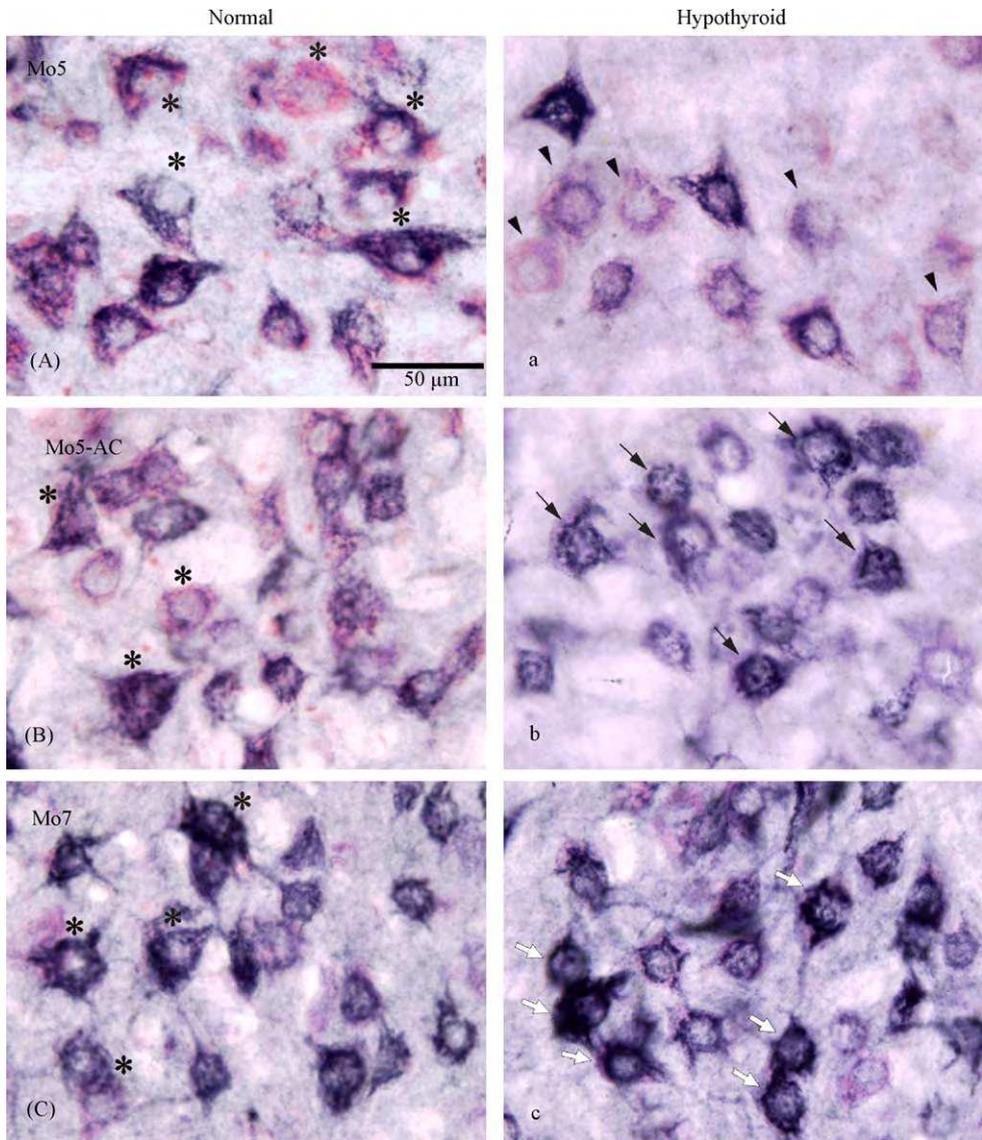


Fig. 7. High-power photomicrographs showing different CGRP immunoreactive intensity in hypothyroid Mo5 nucleus with numerous weakly stained motoneurons (arrowheads, a), and with mostly strong CGRP immunolabeling in Mo5-AC (arrows, b) as well as in Mo7 (white arrows, c). The presence of large immunopositive motoneurons (asterisks) is more detectable in normal motor nuclei compared to their hypothyroid homologues

### 3.3.1 Trigeminal motor nucleus (Mo5)

Although, the number of positive CGRP neurons is gradually increased from strong to moderate and weak in both normal and hypothyroid Mo5 nucleus, the small immunopositive motoneurons had a large proportion (~70%) in hypothyroid pups compared to normal ones (less than 50%). This increase is especially significant for weakly labeled motoneurons ( $P < 0.05$ ). In contrast, the number of the strong, moderate and weakly stained large motoneurons decreased considerably ( $P < 0.01$ ,  $< 0.001$  and  $< 0.01$ , respectively) in comparison with normal motoneurons (see Table 3 a,b and Fig. 7 A,a).

(a) Small motoneurons with diameter  $< 25\mu\text{m}$  and percentage of increase in hypothyroid pups

$\varnothing < 25 \mu\text{m}$	Mo5			Mo5-AC			Mo7		
	Normal	Hypothyroid (%)		Normal	Hypothyroid (%)		Normal	Hypothyroid (%)	
Strong	304 $\pm$ 7.8	354 $\pm$ 4.7	14	12 $\pm$ 0.6	65 $\pm$ 4.4	82**	488 $\pm$ 8.5	812 $\pm$ 25.5	40*
Moderate	848 $\pm$ 22.0	878 $\pm$ 12.2	4	61 $\pm$ 3.5	111 $\pm$ 3.8	45*	1 019 $\pm$ 21.4	1161 $\pm$ 5.9	13
Weak	1015 $\pm$ 25.8	1467 $\pm$ 19.3	31*	68 $\pm$ 2.7	119 $\pm$ 3.2	43*	1 011 $\pm$ 20.6	1255 $\pm$ 5.9	20

(b) Large motoneurons with diameter  $> 25\mu\text{m}$  and percentage of decrease in hypothyroid pups

$\varnothing > 25 \mu\text{m}$	Mo5			Mo5-AC			Mo7		
	Normal	Hypothyroid (%)		Normal	Hypothyroid (%)		Normal	Hypothyroid (%)	
Strong	333 $\pm$ 8.5	170 $\pm$ 2.3	49**	9 $\pm$ 0.6	9 $\pm$ .06	0	132 $\pm$ 17.5	118 $\pm$ 2.9	11
Moderate	921 $\pm$ 16.1	422 $\pm$ 5.9	54**	52 $\pm$ 1.5	15 $\pm$ 1.0	71**	294 $\pm$ 25.1	171 $\pm$ 3.8	42**
Weak	1107 $\pm$ 28.2	661 $\pm$ 9.2	40**	59 $\pm$ 3.3	16 $\pm$ 1.2	73**	294 $\pm$ 27.5	184 $\pm$ 4.4	37**

(c) Percentage of decrease in number of small and large motoneurons devoid of CGRP in hypothyroid pups

Negative	Mo5			Mo5-AC			Mo7		
	Normal	Hypo-thyroid	(%)	Normal	Hypo-thyroid	(%)	Normal	Hypo-thyroid	(%)
$\varnothing < 25 \text{ m}$	181 $\pm$ 3.90	131 $\pm$ 1.33	28*	0	0	0	124 $\pm$ 2.9	72 $\pm$ 1.5	42**
$\varnothing > 25 \text{ m}$	195 $\pm$ 3.2	64 $\pm$ 2.6	67***	0	0	0	33 $\pm$ 0.6	13 $\pm$ 0.3	64***

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$

Table 3. Total number ( $\pm$ S.E.) of CGRP immunolabeled motoneurons with different intensity in each nucleus

### 3.3.2 Trigeminal accessory nucleus (Mo5-AC)

Normal trigeminal accessory motor nucleus showed almost the same pattern of immunolabeling intensity as Mo5 with a proportion of 55% for small motoneurons and 45% for large motoneurons. In the hypothyroid rats, this pattern contained 90% of small immunopositive motoneurons (strong  $P < 0.01$ , and moderate and weak  $P < 0.05$ ) versus 10% of large ones with a significant decrease in moderate and weak intensity ( $P < 0.01$ ) (Table 3 a,b and Fig. 7 B,b).

### 3.3.3 Facial motor nucleus (Mo7)

In comparison with the trigeminal motor nucleus, normal facial motor nucleus showed many more small CGRP-containing motoneurons (>70%) mostly with moderate and weak immunoreactivity. This proportion reached 85% in hypothyroid rats with a significant increase ( $P < 0.05$ ) in the number of small and strongly immunolabeled cells. The proportion of large motoneurons (~20%) in normal weaned pups dropped to ~12% in hypothyroid pups with significant reduction in moderate and weakly labeled motoneurons ( $P < 0.01$ ) (Table 3 a,b and. Fig 7 C,c).

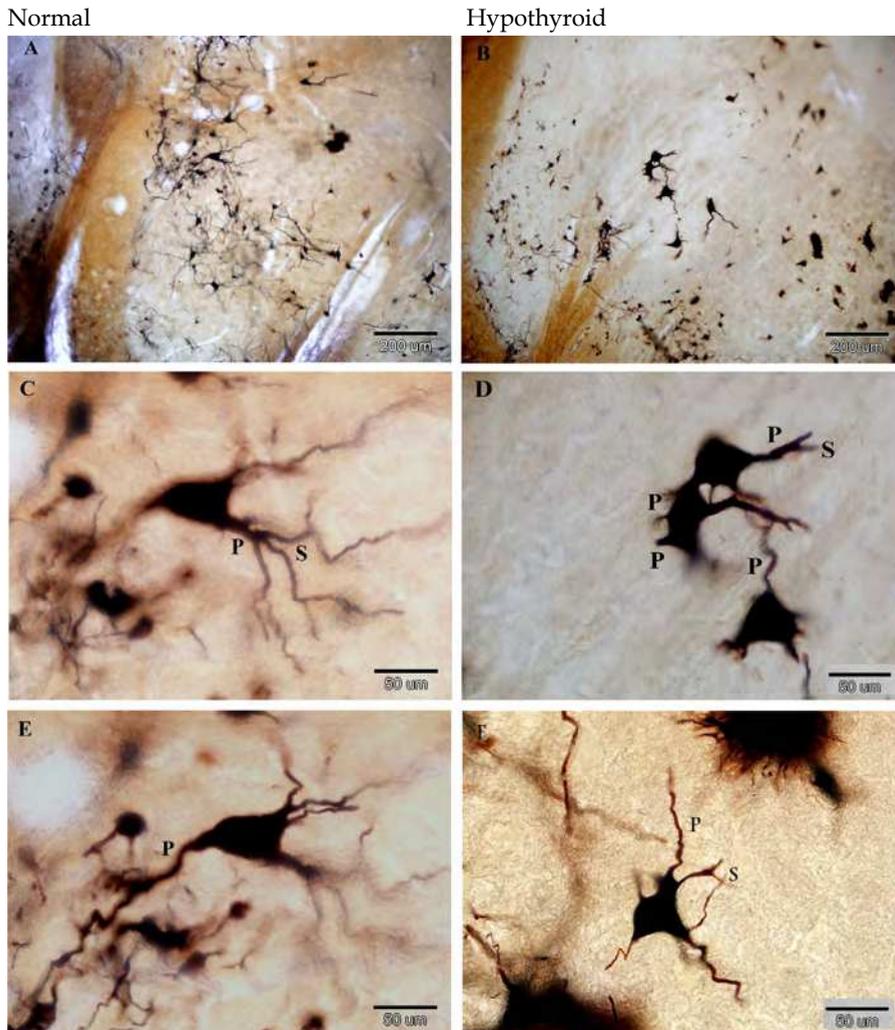


Fig. 8. Golgi staining sections from normal and hypothyroid 23 day old rat pups. Low magnification photomicrographs from Mo5 in normal (A) and hypothyroid (B) pups. Primary (P) and secondary (S) dendrites are shown.

### 3.3.4 Unlabeled motoneurons

In normal pups, about 7% of Mo5 motoneurons and about 5% of Mo7 motoneurons were devoid of CGRP immunostaining, while in the hypothyroid pups, this proportion shifted to 5 and 2%, respectively. In the hypothyroid Mo5, the number of small and large unlabeled neurons was reduced significantly ( $P < 0.05$  and  $<0.001$ ); however, Mo7 nucleus had a lower proportion of large motoneurons ( $P < 0.001$ ) than the small ones ( $P < 0.01$ ). It should be noted that both normal and hypothyroid Mo5-AC nucleus were devoid of unlabeled motoneurons (Table 3c). Nevertheless, no significant difference was observed in the total number of motoneurons in all experimental groups.

### 3.4 Golgi stained motoneurons

The results of cell measuring and counting the primary and secondary dendrites revealed that in hypothyroid pups beside the significant decrease in soma size in trigeminal large motoneurons (a 50% reduction in the number of 900-1200 $\mu\text{m}^2$  motoneurons in PTU-treated group compared to controls,  $P < 0.05$ ), the number of secondary ( $3.8 \pm 0.4$  in PTU-treated compared to  $4.3 \pm 0.5$  in control group) - but not primary dendrites- showed a significant decrease comparing to normal group ( $4.1 \pm 0.3$  vs  $6.5 \pm 0.4$  respectively,  $P < 0.001$ ).

## 4. Conclusion

In the present studies birth weights of hypothyroid animals were slightly lower than normal; this moderate retardation persisted until day 15, then hypothyroid animals stopped growing and clinically became cretinous. The premature profile of masseter muscle begins to appear around the pre-weaning time (day 15) in rats. To meet these muscle functional properties, 2 weeks after birth, the medium-sized labeled motoneurons appeared at the expense of a reduction in the number of small motoneurons. However, during the same period, the number of medium-sized motoneurons was more than 2-fold under the normal values and the quantity of small motoneurons was about 4-fold in hypothyroid pups.

During development hypothyroidism alters the patterns of masseter motoneurons morphology such as soma size, dendritic orientation and arborization pattern and also induces a severe delay in the size transition, which may affect the development and plasticity of oral feeding behavior. On the other hand, immunohistochemical studies in normal animals have shown that motoneurons supplying fast-twitch muscles show a higher level CGRP staining than motoneurons innervating muscles of slow twitch fiber type (Homonko & Theriault, 2000). A severe delay in the appearance of large fast-twitch jaw closing and jaw opening motoneurons due to congenital hypothyroidism suggests an intact thyroid and CGRP state are obligatory for the attainment of normal preadult oro-facial masticatory profile.

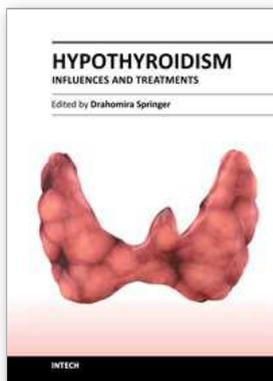
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## **Hypothyroidism - Influences and Treatments**

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Hypothyroidism is the most common thyroid disorder and it is significantly more frequent than presented - millions of people suffer from this disease without knowing it. People with this condition will have symptoms associated with slow metabolism. Estimates of subclinical hypothyroidism range between 3 to 8 %, increasing with age, whereas it more likely affects women than men. About 10% of women may have some degree of thyroid hormone deficiency. Hypothyroidism may affect lipid metabolism, neurological diseases or other clinical conditions. The book includes studies on advancements in diagnosis, regulation and replacement therapy, thyroid ultrasonography and radioiodine therapy for hypothyroidism. "Hypothyroidism - Influences and Treatments" contains many important specifications, results of scientific studies and innovations for endocrine practice.

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