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# Selective Suppression of Neurofilament Antigen Expression in the Hypothyroid Rat Cerebral Cortex

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## SUMMARY

As an integral component of the cytoskeleton neurofilaments play a central role in the establishment and maintenance of neuronal form. In particular, high neurofilament concentrations are characteristic of many classes of axons in the central nervous system. Isolated neurofilaments from rat brain consist of 3 distinct polypeptides with apparent molecular weights 210K, 160K and 68K. A murine monoclonal antibody, mabN210, has been produced which specifically recognizes an epitope associated with the high molecular weight subunit and this antibody has been used to explore the regulation of neurofilament expression during brain development. It has been shown that in the rat cerebellar cortex, the expression of mabN210-immunoreactivity in basket cell axons is severely suppressed in hypothyroidism while neurofilament antigen expression in other cerebellar axons seems not to require thyroid hormones. In view of the well-known cortical deficits in hypothyroidism, these studies have now been extended to include the developing rat cerebral cortex and selected cortical afferent and efferent axons. In hypothyroid rats there is a marked suppression of mabN210-immunoreactivity in the cerebral cortex and corpus callosum and, to a lesser extent, there is a reduction in staining in the internal capsule. By contrast, hypothyroidism did not reduce mabN210-immunoreactivity in the lateral olfactory tract or the stria medullaris. In rats, serum thyroid hormone starts to rise to adult levels on postnatal day 4. It appears that axons that have attained their mature distribution prior to the onset of thyroid hormone

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expression are not affected by hypothyroidism whereas mabN210-immunoreactivity is suppressed in those axonal tracts that reach a mature distribution after P4.

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**Key words:** *Cerebral cortex – Immunocytochemistry – Monoclonal antibodies – Neurofilaments – Thyroid hormone*

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## INTRODUCTION

The rate and extent of development of the central nervous system is modulated by a battery of maternal and fetal hormones. Amongst the better understood of these are the thyroid hormones. Either a lack or an excess of thyroid hormone results in abnormal brain development both in humans and laboratory animals. Congenital hypothyroidism in humans is associated with variable degrees of mental deficiency (Smith et al. 1957) and decreased numbers of cortical neurons (Marinesco 1924; Lotmar 1933; Sanders 1962) and neonatal hyperthyroidism correlates with mental deficiency (Hollingsworth and Mabry 1976; Daneman and Howard 1980) and, on computerized tomographic scans of the brain, ventriculomegaly and exaggerated gyral patterns (Kopelman 1983). Likewise, in rats both hypothyroidism and hyperthyroidism result in impaired learning ability and retarded behavioral development (Eayrs and Lishman 1955; Eayrs and Levine 1963; Eayrs 1964). Histological examination of hypothyroid rats has revealed a decrease in cerebral cortical neuronal size and number, and a decrease in numbers of cerebral cortical axons (Eayrs 1955, 1971), axonal terminals (Cragg 1970), axodendritic processes and synapses (Bass and Young 1973; Ruiz-Marcos et al. 1979, 1982, 1983). In contradistinction to the suppressed neuronal growth of hypothyroidism, hyperthyroid rats have accelerated rates of neuronal proliferation and synaptogenesis but as a consequence of the early termination of neuron production the final result in the adult is similar, decreased brain weight and fewer neurons (Balazs et al. 1971; Nicholson and Altman 1972a,b; Patel et al. 1979; Clos et al. 1982a,b).

At a molecular level, the mechanisms by which thyroid hormone may regulate neuronal maturation are speculative. One possibility is that thyroid hormone acts on the neuronal cytoskeleton to promote neurite extension. Specifically, polymerization of tubulin to form microtubules is essential for extension and elongation of neuronal processes (reviewed in Gozes 1982). Tubulin extracted from brains of hypothyroid rats polymerizes at a markedly reduced rate when compared to tubulin from normal rats. Normal polymerization kinetics can be restored by the addition of microtubule-associated proteins or purified tau protein (Francon et al. 1977; Fellows et al. 1979). Furthermore, the number of microtubule profiles in rat cerebellar Purkinje cells is markedly reduced in hypothyroidism (Faivre et al. 1983).

Neurofilaments are also an integral part of the neuronal cytoskeleton where they are found primarily in the larger diameter axons. Isolated neurofilaments consist of 3 polypeptide subunits with apparent molecular weights of 210, 160 and 68 kdaltons (Liem et al. 1978). By interconnecting with one another, with microtubules and with

other non-cytoskeletal cellular elements neurofilaments form a three-dimensional network that provides structural support for the neurites and thus helps to maintain neuronal form (Lazarides 1980). If hypothyroidism leads to the suppression of microtubule assembly and neurite outgrowth, it is plausible to expect that neurofilament expression may also be suppressed. Such a selective suppression of neurofilament immunoreactivity has been demonstrated in the rat cerebellar cortex using a monoclonal antibody, mabN210, which specifically recognizes an epitope associated with the high molecular weight neurofilament subunit (Leclerc et al. 1985). In the normal cerebellum, neurofilament-associated antigens are expressed in basket cell axons in the molecular layer and by Purkinje cell and mossy fiber axons in the granular layer and the white matter. In hypothyroidism, there is an almost complete suppression of the mabN210 antigen in the basket cell axons while in the mossy fibers and Purkinje cells expression appears unaffected (Leclerc et al. 1985; Gravel et al. manuscript in preparation).

In view of the extensive histological changes observed in the cerebral cortex as a result of abnormal thyroid hormone levels we have extended our observations of neurofilament-associated antigen expression to include the developing cortex and selected cortical afferent and efferent axons from normal, hypothyroid and hyperthyroid rats. In rats, significant levels of thyroid hormones appear in the serum only postnatally. The rise towards adult values starts at postnatal day 4 (P4) reaching a peak for thyroxine at P12 and for tri-iodothyronine at P28 (Dussault and Labrie 1975; Dubois and Dussault 1977). During rat cortical development axonal systems find their synaptic targets at widely-different times. Among the late-developing afferent pathways callosal axons distribute themselves in the contralateral cortex in a mature pattern at P7 (Wise and Jones 1976, 1978; Olavarria and Van Sluyters 1985) and thalamocortical afferents have a mature distribution by P4 to P5 (Lund and Mustari 1977; Wise and Jones 1978). Concerning the cortical efferents, those to the thalamus, the striatum and the pontine nuclei are already in place at birth (Wise et al. 1979) whereas the mature pattern develops in the more distal medullary nuclei and spinal cord grey matter during the first postnatal week (Donatelle 1977; Wise et al. 1979). In contrast, one of the earliest-appearing cortical afferent systems is the lateral olfactory tract (LOT) to the olfactory cortex which has already assumed a mature distribution by E21 (Schwob and Price 1984a,b). We have used the anti-neurofilament monoclonal antibody mabN210 (Leclerc et al. 1985) to study antigen expression in axons of the developing cerebral cortex in hypothyroid and hyperthyroid neonatal rats with particular attention to selective effects on different axonal systems.

## MATERIALS AND METHODS

### *Immunocytochemistry*

The production and characterization of monoclonal antibody mabN210 has been described previously (Leclerc et al. 1985). P16 and P25 rats were deeply anaesthetized with sodium pentobarbital. After surgical exposure of the heart, each animal was perfused via the left ventricle with ice-cold 4% paraformaldehyde/0.2% glutaraldehyde in PB (0.1 M phosphate buffer, pH 7.4) with a volume ranging from 25 to 60 ml

depending on the size of the animal. The brain was postfixed overnight in 4% paraformaldehyde in PB alone. Sections were cut coronally at 50  $\mu\text{m}$  using a freezing-stage microtome.

To detect specific immunoreactivity, sections were incubated in monoclonal antibody overnight. In all of the examples shown here, mabN210 was used directly in spent culture medium diluted 1:8 into 10% normal horse serum in PBS (0.1 M phosphate buffer, 0.15 M NaCl, pH 7.4). To detect specific antibody binding, sections were subsequently incubated 2 h in rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase (Dako Inc.) diluted 1:100 in 10% normal horse serum. Antibody binding was revealed using 4-chloro-1-naphthol as substrate (Hawkes et al. 1982). The sections were washed for 15 min in several changes of PBS between incubations. Sections in which the primary antibody was omitted or replaced by myeloma-conditioned medium gave no staining.

### *Hypothyroidism*

Sprague-Dawley rats were rendered hypothyroid by inclusion of 0.05% w/v *n*-propyl-2-thiouracil (PTU; Sigma Inc.) and 10% w/v sucrose in the maternal drinking water (Dussault and Walker 1978). Sucrose is required to make the solution palatable. Treatment commenced prenatally at the 16th day of gestation by treatment of the maternal drinking water and was continued throughout the postnatal life. The pups remained with their mothers throughout the treatment period. PTU is a reliable agent in producing hypothyroidism (Legrand 1983) and can be monitored by observing overt signs such as reduced body weight, decreased locomotor activities and scarce fur. Control animals received the sucrose solution alone.

### *Hyperthyroidism*

In order to avoid endogenous thyroid hormone production from affecting serum thyroid hormone levels, rats that were treated with PTU were rendered hyperthyroid by receiving daily subcutaneous injections of L-thyroxine (T<sub>4</sub>; Sigma Inc.). Doses ranged from 0.20 to 1.25  $\mu\text{g}$  from birth to P25. The per weight dose was on a sliding scale determined by the age of the rat and corresponded to 5 times the minimal dose required to correct all of the cerebellar structural abnormalities of PTU-treated rats (Rabie et al. 1979). These same doses are the minimum ones necessary to produce hyperthyroid characteristics on cerebellar histology (Rabie et al. 1977).

### *Photography*

All of the brain areas studied were photographed using a Zeiss light microscope. In order to have comparable photographic images, all pictures were taken with the same exposure time and filter settings and all prints were made using identical printing techniques.

The cingulate cortex is located 7.0 mm anterior to the interaural line, according to the atlas of Paxinos and Watson (1982). This cortical area corresponds to area 24 (Caviness 1975) and to the ventral anterior cingulate cortex (Krettek and Price 1977). The corpus callosum was photographed at this same level immediately below the

interhemispheric fissure. The internal capsule and the stria medullaris were also photographed at this level. Photographs of the lateral olfactory tract were taken from sections 9.2 mm anterior to the interaural line. Primary sensory (area 3), primary motor (area 6) and primary visual (area 17) cortical areas were according to Caviness (1975).

## RESULTS

PTU is a synthetic antithyroid compound which blocks intrathyroidal organification of iodine and inhibits extrathyroidal monodeiodination of T4 to tri-iodothyronine (Oppenheimer et al. 1972). Hypothyroid rat pups were overtly different from their control littermates. The PTU-treated rats were much smaller, at P16 weighing an average of 18.5 g as compared to 32.5 g for the controls, had decreased locomotor ability and scarce fur, all signs of hypothyroidism. Moreover, histological examination of the cerebellar cortex of the treated rats revealed typical morphology associated with hypothyroidism (Legrand 1983; Leclerc et al. 1985); Purkinje cell dendritic hypoplasia, delayed appearance of the molecular layer and suppressed basket cell axonal mabN210 immunoreactivity (not illustrated). To distinguish thyroid hormone deficiency from hormone-independent PTU toxicity, one group of control rats was treated with both PTU and remedial doses of T4. Administration of thyroxine to PTU-treated rats prevented the somatic changes and the histological findings in the cerebellum (not illustrated) and cerebrum (see below) were normal, thus indicating that the changes in the PTU-treated rats were due to thyroid hormone deficiency rather than direct PTU toxicity. The doses of T4 used in the hyperthyroid regime were chosen as being sufficient to decrease both cerebellar DNA content (Rabie et al. 1977) and the number of cerebellar neurons (Rabie et al. 1977, Clos et al. 1982a, b) and to accelerate the maturation of the cerebellar cortex (Clos et al. 1974).

Monoclonal antibody mabN210 is secreted by a murine hybridoma constructed from a mouse immunized with neonatal rat cerebellar homogenate (Leclerc et al. 1985). On Western blots it recognizes the high molecular weight neurofilament subunit only, and by immunocytochemistry it stains neurofilament-rich axons in the adult and developing rat cerebellar cortex. When used to immunoperoxidase stain the adult rat cortex, mabN210 produces a characteristic laminar distribution of reaction product. Figure 1A illustrates the distribution of immunoreactivity in the adult cingulate cortex, labelled according to Krettek and Price (1977). Reaction product is concentrated preferentially in layers I, III and VI. Layer II is only sparsely immunoreactive. Layer V is clearly subdivisible into two, a more superficial sublayer with little reaction product and a wider, more-strongly stained sublayer to the interior. Using autoradiographic tracing techniques, Krettek and Price (1977) did not distinguish a layer IV in the rat ventral anterior cingulate cortex. However, earlier authors using fiber stains (e.g. Krieg 1946), split lamina V into laminae IV and V and it seems likely that the unreactive sublayer identified in layer V by mabN210 immunocytochemistry corresponds to lamina IV. As in the cerebellar cortex, reaction product is associated with axon profiles and neither dendrites nor non-neuronal cell types are immunoreactive. The axonal location of the staining is illustrated at higher magnification in Fig. 1B which shows a

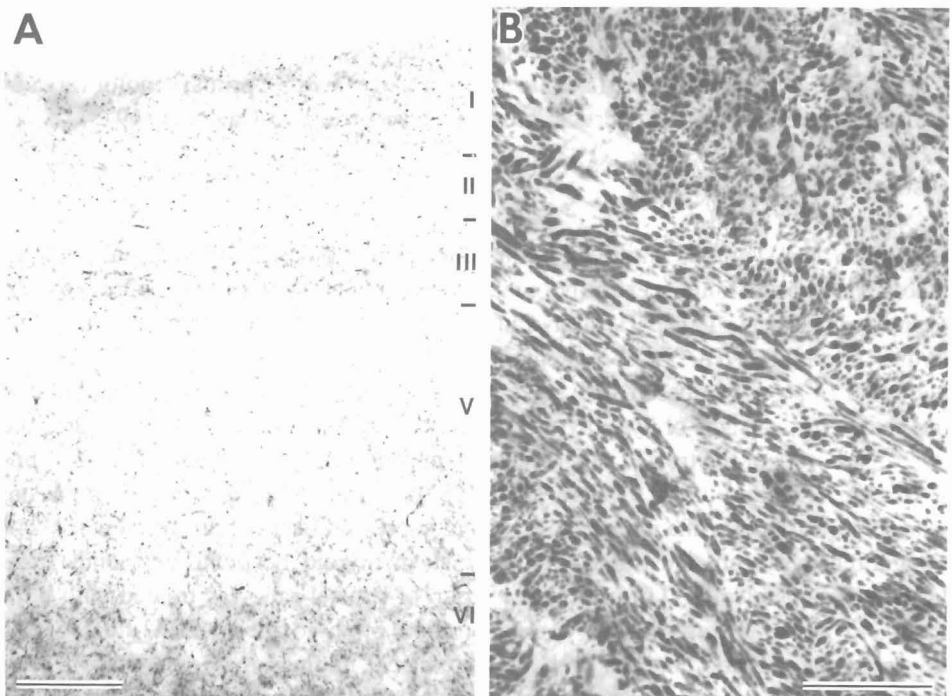


Fig. 1. *A*: A coronal section through the adult rat ventral anterior cingulate cortex immunoperoxidase stained with mabN210. Reaction product has a laminar distribution with layers I, III and VI more intensely stained than the others. According to Krettek and Price (1977), there is no layer IV in rat ventral anterior cingulate cortex: however, mabN210 clearly reveals a line of demarcation dividing layer V into a superficial, weakly-reactive sublayer and a more strongly immunoreactive deeper sublayer. Scale bar = 100  $\mu$ m.

*B*: A mabN210-stained section through the adult internal capsule showing immunoreactive axon profiles. The reaction product is associated with the cores of the axons. Scale bar = 30  $\mu$ m.

section through the internal capsule: reaction product is deposited in the cores of the axons not in the surrounding myelin sheaths.

MabN210 has been used here to study the thyroid hormone sensitivity of expression of a well-characterized polypeptide-associated antigen in the rat cerebral cortex. In adult rats the major afferent systems to the cingulate cortex are from thalamic nuclei and the contralateral cortex. The thalamocortical afferents, primarily from the mediodorsal nucleus, are distributed preferentially in layers I and III (Krettek and Price 1977). Corticocortical afferents are distributed throughout the depth of the cortex, but are concentrated preferentially in layers I and III (Beckstead 1979). To illustrate the consequences of abnormal T4 levels for cortical maturation Fig. 2 contrasts the distribution of the mabN210-antigen in immunoperoxidase-stained sections of the upper layers of the ventral anterior cingulate cortex in hypothyroid, control and hyperthyroid rats aged P16 and P25. In the control animals, layers I and III are more strongly labeled, reflecting the greater density of immunoreactive axons. As in the adult, only axons are labeled with no staining of neuronal cell bodies, glia or endothelial cells. The

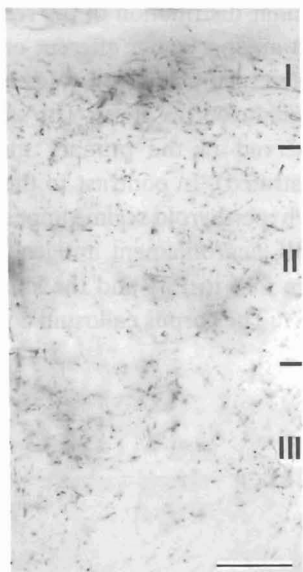
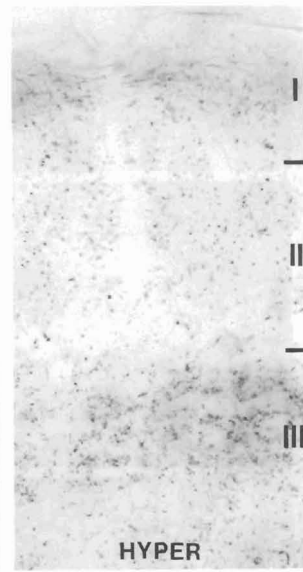
**P16****P25**

Fig. 2. Coronal sections of neonatal rat ventral anterior cingulate cortex immunoperoxidase stained with mabN210. Hypothyroid (HYPO), control (CTL) and hyperthyroid (HYPER) animals are illustrated at P16 and P25. There is marked suppression of mabN210 immunoreactivity in the hypothyroid animals as compared to the controls. There is no difference between hyperthyroid and control animals. The greater axonal concentration in layers I and III is evident. Scale bar = 50  $\mu$ m.

density of the reaction product deposits increases between P16 and P25 but there is no qualitative change in their laminar distribution. The effects of neonatal hypothyroidism are dramatic. There is a severe suppression of mabN210-immunoreactivity throughout all layers of the anterior cingulate cortex in the hypothyroid rats at both ages. From the

laminar distribution of the residual mabN210-immunoreactivity there is no suggestion that any particular afferent or efferent cortical axonal systems or cortical laminae are preferentially affected. Neurofilament antigen suppression was not peculiar to the cingulate cortex alone. The same suppression of mabN210-immunoreactivity was also observed in the primary motor, sensory and visual cerebral cortical areas (not illustrated). In contrast to the dramatic consequences of thyroid hormone deficiency, the hyperthyroid regime appears to have no effect on the intracortical expression of the 210K neurofilament antigen. The intensity of mabN210-staining increased normally from P16 to P25 and the laminar distribution was unchanged.

The corpus callosum contains the corticocortical interhemispheric axons. During

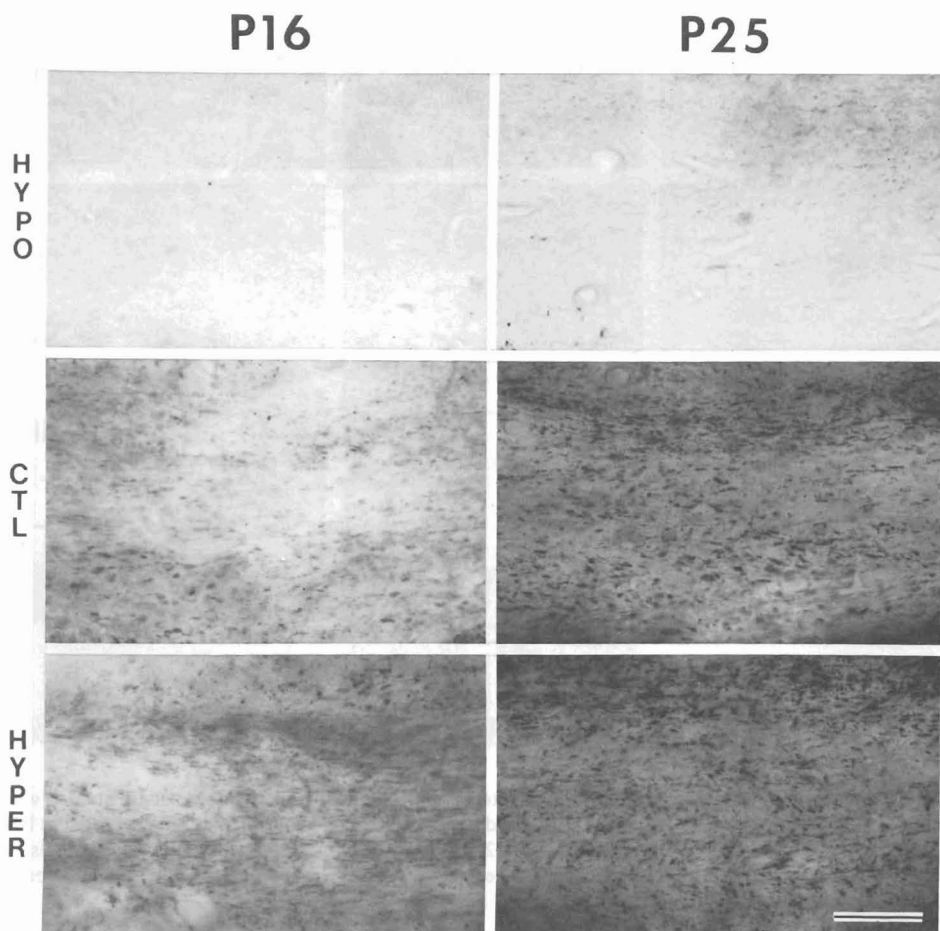


Fig. 3. Coronal sections of neonatal rat corpus callosum immunoperoxidase-stained with mabN210. Hypothyroid (HYPO), control (CTL) and hyperthyroid (HYPER) animals are illustrated at P16 and P25. There is marked suppression of mabN210-immunoreactivity in the hypothyroid animals as compared to the controls, with no difference between the hyperthyroid and controls. There is an increase in neurofilament reactivity in both control and hyperthyroid animals from P16 to P25. Scale bar = 50  $\mu$ m.

development the first axons cross the midline between gestational ages E18 and E19 (Floeter and Jones 1985). Subsequently, commissural axons grow into the cortical plate between days P4 and P6 assuming an adult pattern of distribution on P7 (Wise and Jones 1976, 1978; Olavarria and Van Sluyters 1985). Figure 3 compares the corpus callosum in normal, hypothyroid and hyperthyroid rats. In the control animals immunoreactive axon profiles are found throughout the corpus callosum. The staining intensity increases between P16 and P25. Callosal mabN210-immunoreactivity is highly sensitive to hypothyroidism. At both P16 and P25 there is an almost complete suppression of mabN210-staining in the corpus callosum of hypothyroid rats with

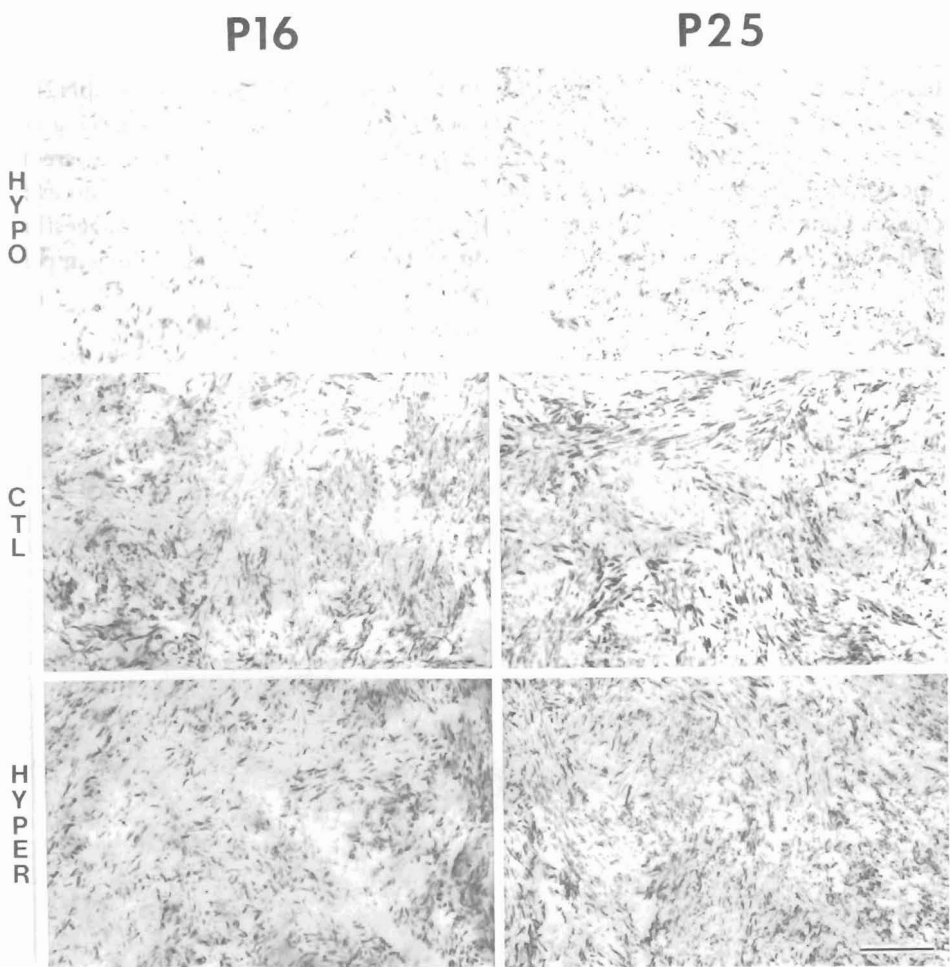


Fig. 4. Coronal sections of neonatal rat internal capsule immunoperoxidase-stained with mabN210. Hypothyroid (HYPO), control (CTL) and hyperthyroid (HYPER) animals are illustrated at P16 and P25. There is marked suppression of mabN210-immunoreactivity in the hypothyroid animals as compared to the controls, but no difference between the hyperthyroid and control animals. The stained axons have a smaller caliber in the hypothyroid animals as compared to the control and hyperthyroid ones. Scale bar = 50  $\mu$ m.

scarcely any axons remaining immunoreactive. As was seen for the intracortical immunoreactivity, hyperthyroidism had no effect on either the intensity or the distribution of callosal staining.

The internal capsule consists primarily of a mixture of thalamic afferents to the cerebral cortex together with a subset of the cortical efferents. During cortical development the arrival of the thalamocortical afferents precedes the appearance of commissural axons. At E18 many have already arrived in the subcortical white matter where they will occupy the intermediate zone until they invade the cortical plate between P2 and P3 to assume the adult distribution between P4 and P5 (Lund and Mustari 1977; Wise and Jones 1978). The corticothalamic, corticostriatal and corticopontine efferents are distributed to their targets via the internal capsule at P1 and cortical efferents to the more distal targets develop during the first postnatal week (Donatelle 1977; Wise et al. 1979). Figure 4 shows that mabN210-immunoreactive axons are found throughout the internal capsule. In the hypothyroid rats there is again suppression of mabN210-immunoreactivity with no difference between control and hyperthyroid animals (Fig. 3). However, the suppression of the neurofilament antigen expression is not as severe as in the corpus callosum and some residual limited immunoreactivity remains and even increases somewhat between P16 and P25. In the hypothyroid rats there is a suggestion that the internal capsular axons are smaller in caliber, thus possibly accounting in part for the reduction in mabN210-immunoreactivity.

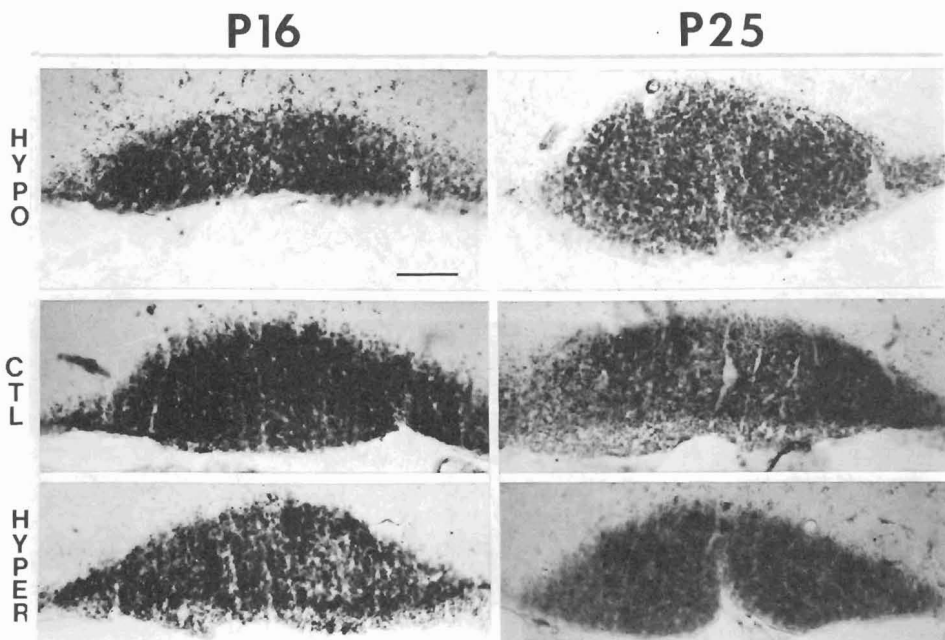


Fig. 5. Coronal sections of neonatal rat lateral olfactory tract immunoperoxidase-stained with mabN210. Hypothyroid (HYPO), control (CTL) and hyperthyroid (HYPER) animals are illustrated at P16 and P25. There is no appreciable difference in mabN210-immunoreactivity between the hypothyroid, control and hyperthyroid animals. Scale bar = 100  $\mu$ m.

The axons of the lateral olfactory tract (LOT) are amongst the earliest to develop, being in place by E16 and extending throughout layer I of the olfactory cortex in a mature pattern by E21 (Schwob and Price 1984a,b). On the day of birth electrical stimulation of the LOT elicits olfactory cortical evoked responses thus attesting to the early functioning synaptic connections of the LOT (Schwob et al. 1984). MabN210-immunoreactivity in the LOT is extremely intense in normal animals at P16, diminishing somewhat by P25 but still remaining one of the most-intensely mabN210-immunoreactive tracts. In stark contrast to the axons within the cortex, the corpus callosum and the internal capsule, the mabN210-immunoreactivity in the LOT is insensitive to hypothyroidism. Strongly-immunoreactive axon profiles are seen throughout the tract at both P16 and P25 with no sign of a reduction in either number of stained profiles or staining intensity (Fig. 5). In hyperthyroid animals the LOT staining was indistinguishable from that in the controls.

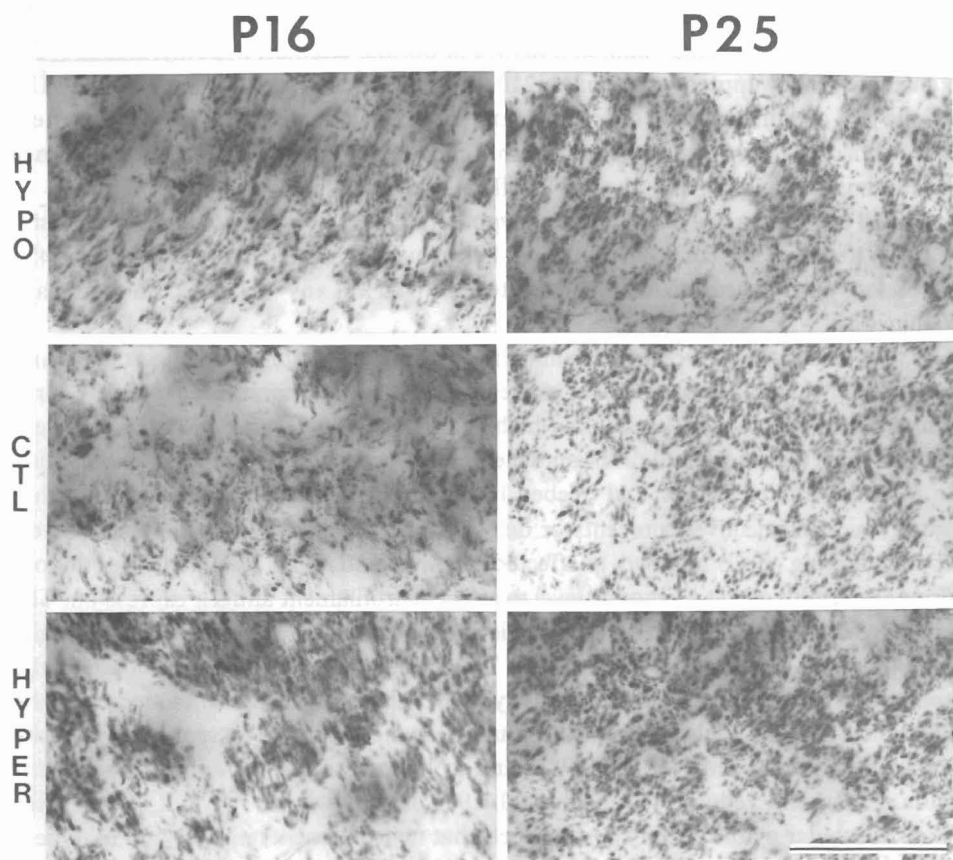


Fig. 6. Coronal sections of neonatal rat stria medullaris immunoperoxidase-stained with mabN210. Hypothyroid (HYPO), control (CTL) and hyperthyroid (HYPER) animals are illustrated at P16 and P25. There is no appreciable difference in mabN210-immunoreactivity between the hypothyroid, control and hyperthyroid animals. Scale = 50  $\mu$ m.

Another early-formed fiber tract, the stria medullaris (SM), contains axons projecting from the amygdalar area to the habenular nuclei. The SM is present as an identifiable structure by E14.5 and by E15.5 fully extends to the habenular nuclei (Marchand and Blanchet 1985). The mabN210-immunoreactivity in the SM resembles that in the LOT and was affected by neither hypothyroidism nor hyperthyroidism (Fig. 6).

## DISCUSSION

Many axons of the cerebral cortex and its afferent and efferent pathways display high levels of mabN210-immunoreactivity. This correlates well with the dense accumulations of neurofilament profiles seen in many axons by electron microscopy. Immunoreactivity was always specifically associated with axon profiles: neuronal cell bodies, dendrites and non-neuronal elements were unreactive. The present results demonstrate that hypothyroidism markedly, but selectively, suppresses mabN210 immunoreactivity in neonatal rats. In the axons of the cerebral cortex and corpus callosum, both sets strongly immunoreactive in normal animals, hypothyroidism leads to an almost total suppression of mabN210-antigen expression. By contrast, in the LOT and the SM the intensity and distribution of mabN210-immunoreactivity is unaffected. The effects of hypothyroidism on the internal capsule are intermediate in severity. Since T4 administration prevents the PTU effect, we have attributed the inhibition of mabN210 expression to a direct effect of hypothyroidism rather than to an indirect result of PTU toxicity. By contrast, the mild hyperthyroid regime had no effect on the expression of the neurofilament-associated antigen in any of the axon pathways examined.

It would not be correct to assume that because the mabN210-antigen is thyroid hormone sensitive all neurofilament expression has been suppressed. Isolated neurofilaments are composed of 3 subunits which attain their adult levels of expression at very different rates during normal rat brain development with the 210 kdalton subunit being the most protracted (Shaw and Weber 1982). From our results, it is possible that hypothyroidism selectively prevents or delays the appearance of only the 210 kdalton subunit, leaving the other two unaffected. Nor should it be assumed that thyroid hormones act directly at a genomic level to affect neurofilament antigen expression. The hypothyroid rats exhibit extensive somatic abnormalities so the effects on neuronal development could be extremely indirect. There is some evidence suggesting that the suppression of mabN210-immunoreactivity in hypothyroidism may be secondary to the inhibition of microtubule assembly. Tubulin polymerization is essential for neurite extension and elongation (Gozes 1982) and in hypothyroidism the rate of polymerization is markedly reduced (Francon et al. 1977; Fellows et al. 1979). It is only after the microtubular cytoskeleton is established that neurofilaments appear and assume a structural role (Lazarides 1980; Shaw and Weber 1982) so that interference with microtubule assembly in hypothyroid rats could block the progression of normal axonal maturation and thus secondarily affect neurofilament expression. Axon tracts such as the LOT and SM which mature well before the appearance of serum thyroid hormone would thus be insensitive to its absence.

It is unlikely that the marked suppression of mabN210-immunoreactivity in the hypothalamic cerebral cortex and corpus callosum is due simply to a lack of axons. Using quantitative silver staining methods, Eayrs (1955, 1971) showed that in hypothyroid rats there is a 20–25% decrease in the number of axons throughout the cortical layers with a greater decrease of 36% in layer IV. A similar reduction was found in the number of axonal terminals per cortical neuron in hypothyroid rats using quantitative electron-microscopic techniques (Cragg et al. 1970). A 20% decrease in the total number of axons cannot account for the severity of the observed changes. Furthermore, there is no obvious reduction in the corpus callosum cross-sectional area. Likewise, although the suppression of mabN210-immunoreactivity within the internal capsule of the hypothyroid rats seems to be accompanied with decreased caliber of axons (as a general rule axonal neurofilament content is proportional to axonal diameter e.g. Friede and Samorajski 1970; Weiss and Mayr 1971; Berthold 1978), diminished axonal caliber alone cannot explain the widespread suppression of mabN210-immunoreactivity since the remaining unstained axons in the hypothyroid cerebral cortex and corpus callosum did not seem to differ in caliber from those of the control and hyperthyroid rats.

A possible explanation of the selective effects of hypothyroidism lies in the correlation between the sensitivity to hypothyroidism and the age at which an axon tract assumes its mature disposition. The LOT and SM are axon pathways which appear and establish synaptic contact with their targets much earlier than the axons in the corpus callosum or internal capsule. MabN210-immunoreactivity in these early-maturing tracts was not affected by hypothyroidism while in a late-maturing pathway, the corpus callosum, antigen suppression was the most severe. Thus, it appears plausible that the time of axonal maturation is critical to whether axonal neurofilament immunoreactivity will be suppressed by hypothyroidism. In normal rats serum thyroid hormone levels start climbing towards adult values on P4. Axonal systems which are already in situ before P4, such as the LOT and SM, are insensitive to hypothyroidism. The fibers of the corpus callosum assume a mature distribution at P7 and are the most severely affected by hypothyroidism. The results obtained from the internal capsule are consistent with this interpretation. With a mixed population of early and late-maturing axons it is predicted that the effects of hypothyroidism be intermediate between those in the corpus callosum and the LOT, with immunoreactivity abolished in some axons and not in others. This model is consistent with data obtained from the developing cerebellar cortex where mabN210-immunoreactivity is almost completely suppressed by hypothyroidism in the late-maturing basket cell axons but much less affected in the earlier-appearing Purkinje cell and mossy fiber axons (Leclerc et al. 1985; Gravel et al. manuscript in preparation). It must be underlined that there is no reason to suppose that thyroid hormone acts at the genomic level to directly suppress the expression of the mabN210-epitope. Axonogenesis is a complex process with numerous successive stages and the expression of the mabN210-epitope lies near the end of this sequence (e.g. Leclerc et al. 1985). Thus, any thyroid hormone-sensitive step along the way would inevitably result in the mabN210-epitope suppression found in hypothyroidism.

It is not known if similar antigenic changes are correlated with hypothyroidism in humans. However, it has been confirmed that mabN210 does stain axons in adult

human brain tissue (unpublished observations) so, given that neurofilament immunoreactivity survives prolonged periods of fixation (Majocho et al. 1985), mabN210 may prove to be a valuable probe to study the effects of hypothyroidism on human cerebral corticogenesis and axonogenesis using available libraries of human pathologic material.

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