Thyroid Hormones in Brain Development and Function

Last Updated: December 17, 2012

Authors

Juan Bernal, M.D., Ph.D.
Professor of Research Instituto de Investigaciones Biomedicas, CSIC-UAM Center for Biomedical Research On Rare Diseases (CIBERER) Arturo Duperier 4, 28029 Madrid, Spain

INTRODUCTION

Thyroid hormones are essential for brain maturation, and for brain function throughout life. In adults, thyroid diseases can lead to various clinical manifestations (1, 2). For example, hypothyroidism causes lethargy, hyporeflexia and poor motor coordination. Subclinical hypothyroidism is often associated with memory impairment. Hypothyroidism is also associated to bipolar affective disorders, depression, or loss of cognitive functions, especially in the elderly (3). Hyperthyroidism causes anxiety, irritability, and hyperreflexia. Both, hypothyroidism or hyperthyroidism can lead to mood disorders, dementia, confusion, and personality changes. Most of these disorders are usually reversible with proper treatment, indicating that thyroid hormone alterations of adult onset do not leave permanent structural defects.

The actions of thyroid hormone during development are different, in the sense that they are required to perform certain actions during specific time windows. Thyroid hormone deficiency, even of short duration may lead to irreversible brain damage, the consequences of which depend on the specific timing of onset and duration of thyroid hormone deficiency (4-8).

The rat has been the most widely used animal model in the sudy of thyroid physiology and in the actions of thyroid hormone in brain, and the use of gene knock out and knock in mice is providing new insights. When the results of animal studies are extrapolated to the human, it is important to realize that the timing of development in relation to birth among mammals presents substantial differences, even if the sequence of events is similar (9-11). Most brain growth occurs after birth in man and in rodents, but rats and mice are born with a less developed thyroid axis than humans. As a useful reference “the newborn rat may be compared with a human fetus in the second trimester of pregnancy, and the newborn human baby to a 6-10 day old rat” (12).

EFFECTS OF THYROID HORMONE ON BRAIN DEVELOPMENTAL PROCESSES

Structural Defects Caused by Hypothyroidism

The hypothyroid brain presents many structural defects (for an extensive review see (12):- 1) increases in cell density in the cerebral cortex, due to reduction of the neuropil (13, 14), 2) lower cell numbers in regions with significant postnatal cell acquisitions, for example the olfactory bulb and the granular layers of the hippocampus and cerebellum (14, 15), 3) decreased number of GABAergic interneurons in the cerebellum with accumulation of neuronal precursors (16), and 4) reduced number of interneurons

in the cerebral cortex (17). Some specific cell types display stunted dendritic and / or axonal growth and maturation, for example cholinergic cells (18, 19), Purkinje cells of the cerebellum (Fig 1) (20, 21) and pyramidal cells of cortex layer V (22, 23). Changes of dendritic spine number are also observed in the cortex and hippocampus after adult onset hypothyroidism, and are reversible with thyroxine treatment (24, 25). These morphological changes are a consequence of altered biological processes as follows.

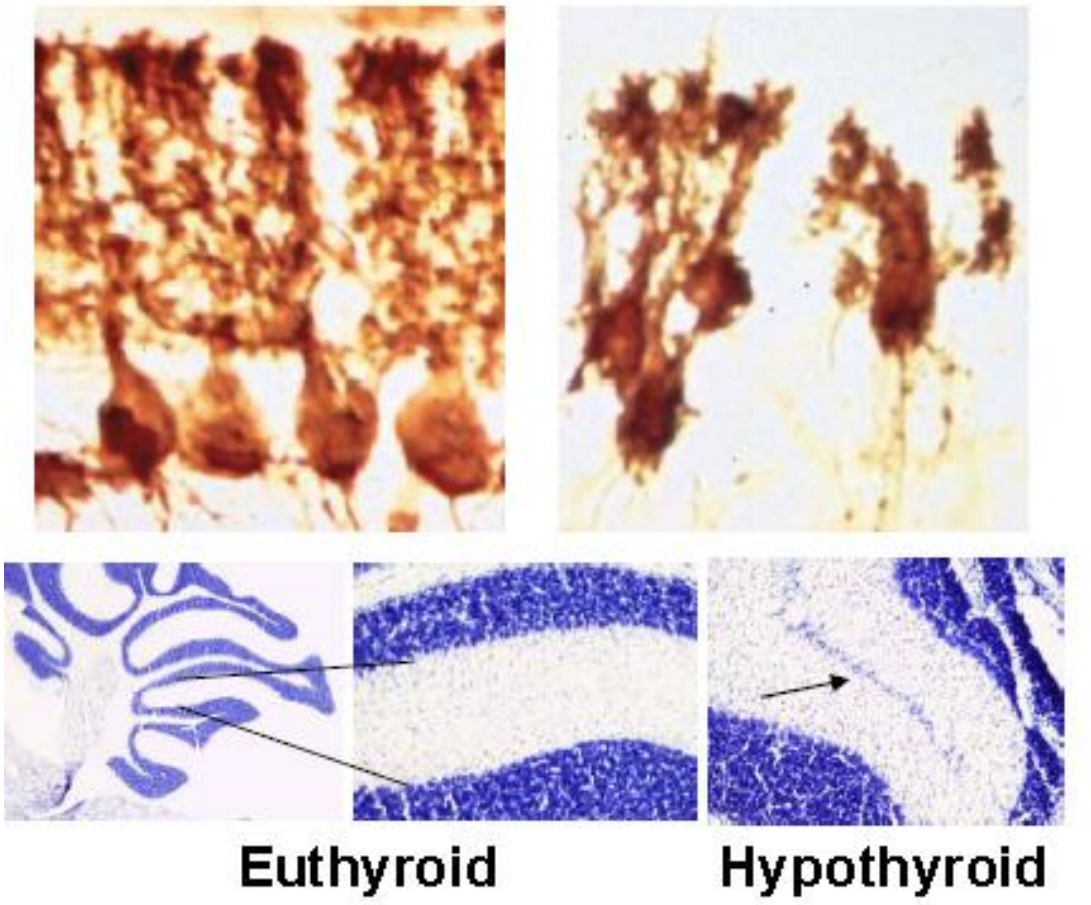


Fig 1.-Postnatal morphological changes in the rodent cerebellum after neonatal hypothyroidism. Upper panel: Purkinje cells in a normal (left) and hypothyroid rat (right). Lower panel: persistence of the external granular layer (arrow) in a hypothyroid mouse cerebellum.

Neurogenesis

Thyroid hormones are involved in late events of neural development, such as migration and terminal differentiation of neurons and glia. A possible role of thyroid hormones on proliferation and differentiation of neural precursors in the embryonic neurogenic areas has been shown in a few cases in relation to the effects of maternal thyroid hormones (26, 27), and in tadpoles during premetamorphosis (28). Most studies concern the effects of thyroid hormone on neurogenesis in adult animals. Adult neurogenesis occurs in two regions: the subventricular zone (SVZ), and the subgranular zone (SGZ). The SVZ, located below the surface of the lateral ventricles, generates olfactory bulb

interneurons in adult rodents. The SGZ is adjacent to the granular layer of the dentate gyrus, and generates granular neurons. Hypothyroidism depresses, and thyroid hormone administration stimulates, neurogenesis in these two areas (29-32). In the SVZ T3 increases differentiation of neuronal precursors (33) and acts through TRa1 to increase the commitment of neural stem cells to migrating neuroblasts. This action correlates with repression of the Sox2 gene (34). The effects of T3 on embryonic and adult neurogenesis appear to be mediated predominantly by TRα1 (26, 28, 34). However, effects of TRβ have also been shown (35).

Cell Migration

Thyroid hormone exerts important influences on cell migration in the cerebral cortex, hippocampus and cerebellum (Fig 1). Among relevant possible mechanisms is the action on the radial glia, one of the first differentiated cells generated in the neuroepithelium. The radial glia extends long processes to the cerebral wall, providing a scaffold that serves for cell migration (36, 37). Later in development, the radial glia differentiates into astrocytes and ependymal cells (38). Radial glia maturation in the fetal rat brain is delayed in the hippocampus of hypothyroid rats (39).

In the cerebral cortex, thyroid hormones are needed for the proper arrangement of the six layer pattern, formed by the timely migration of cells originated in the ventricular neuroepithelium. Deficiency of thyroid hormone during the period of cortical development leads to less defined cortical layers, due to disturbances of cell migration (40-42). One mechanism by which thyroid hormones may influence neuronal migration in the cerebral cortex is through the regulation of the expression of the *Reln* gene (Fig 2). The product of this gene, Reelin, is an extracellular matrix protein produced by neurons located in layer I of the cerebral cortex, the Cajal-Retzius cells (43). Thyroid hormone regulates the expression of at least two genes expressed by these cells, *Reln*, and *Ptgds* (encoding Prostaglandin D2 synthase) (44, 45). Reelin is essential for the orderly migration and the establishment of neocortical layers. Cajal-Retzius cells have also an important role in the migration of neuronal precursors in the hippocampus and in the establishment of synaptic connections (46).

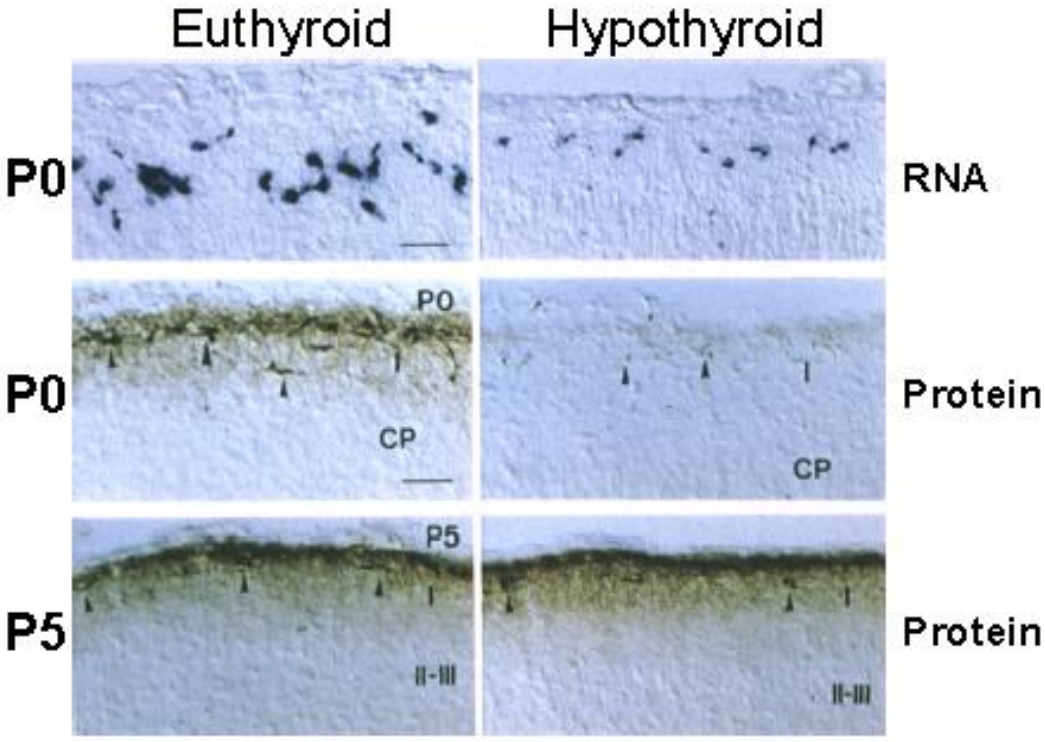


Fig 2.-Regulation of Reelin by thyroid hormone. The figure shows slices of the cerebral cortex from euthyroid and hypothyroid newborn rats. The upper panels show in situ hybridization for Reelin RNA. The midle and lower panels show immunohistochemistry of Reelin protein. The RNA is present in isolated cells of layer I, known as Cajal-Retzius cells. The RNA is very low in the hypothyroid animals. The Reelin protein is a matrix protein, seen in these slices concentrated also in layer I. On P0 there is no Reelin protein in the hypothyroid animals, but on P5, the amount of Reelin protein is normal (from Alvarez-Dolado M et al, J Neurosci. 19:6979-3, 1999).

Neuronal migration in the cerebral cortex is extremely sensitive to thyroid hormones, and even minor deficiencies are associated with migration defects. For example, transient maternal hypothyroidism in pregnant rats at embryonic days 12 to 15 caused significant misplacement of cells in the neocortex and hippocampus of the offspring, when analyzed at 40 days of age, and audiogenic seizures (47). Moderate thyroid hormone deficiency during pregnancy caused neuronal ectopias in the corpus callosum (48).

In the cerebellum thyroid hormones are involved in the late phase of granular cell migration from the external germinal layer to the internal granular layer. This process takes place postnatally in rodents and is completed by P20, when the EGL disappears. Proliferating granular cells migrate through the molecular layer, along the fibers of Bergmann glial cells, when they exit the cell cycle. The main factor for this control is Sonic Hedgehog, produced by the Purkinje cells. It is likely that thyroid hormones are one additional factor controlling cell division. A striking and very characteristic feature of the hypothyroid cerebellum is a delay in the migration of granule cells so that the EGL persists beyond P20 (Fig 1) (49).

Myelination

Hypothyroidism causes delayed and poor deposition of myelin (50-52) whereas hyperthyroidism accelerates myelination (53). After prolonged neonatal hypothyroidism, the number of myelinated axons in adult rats is abnormally low in hypothyroid animals although most of the myelinated axons appear to have a normal thickness of the myelin sheath (fig 3). Thyroid hormones exert important effects on differentiation of oligodendrocytes, the cells that produce myelin. During development, hypothyroidism delays oligodendrocyte differentiation and myelin gene expression, eventually becoming normal even in the absence of thyroid hormone treatment. However, the myelination defect remains in adult animals, although oligodendrocytes are not targets of thyroid hormones in the adult. It is likely that one additional factor might be indirect effects through axonal maturation, which is impaired in hypothyroidism (19, 42). The lower diameter of axons in hypothyroid animals would prevent myelination of small axons, with normal myelination of only those axons that reach a critical size (54).

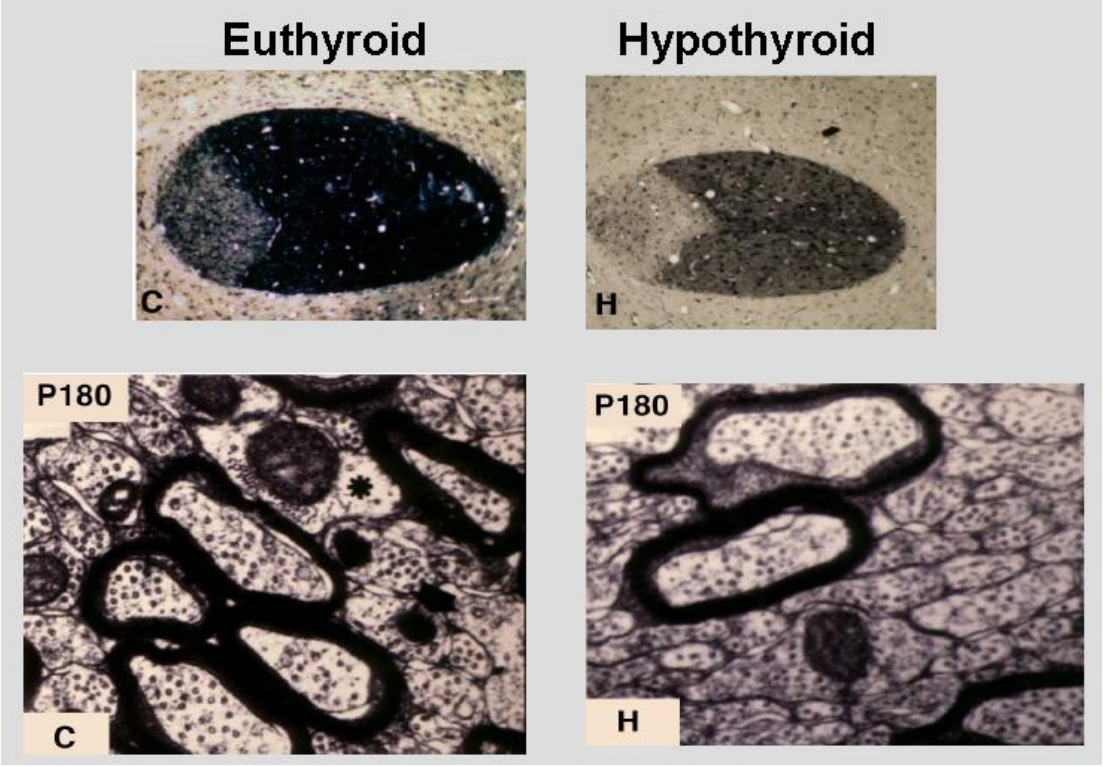
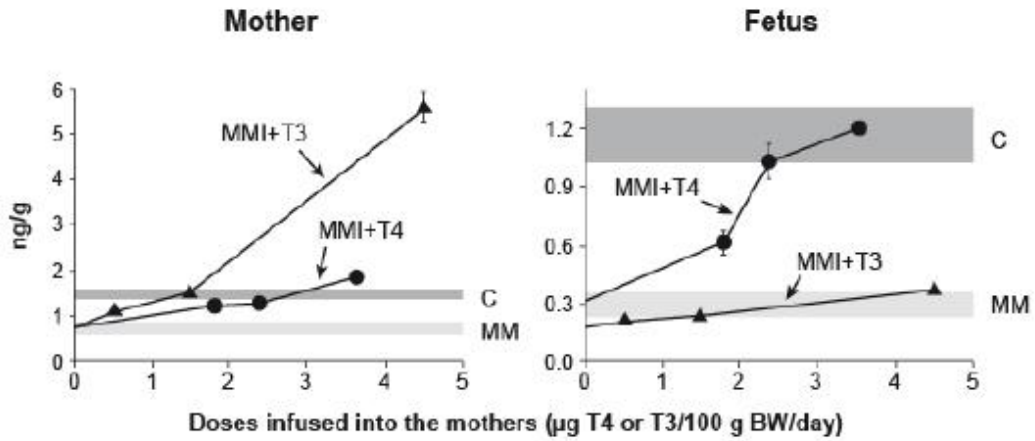


Fig 3.-Myelination in the anterior commisure of euthyroid and hypothyroid rats. Hypothyroidism was produced during the neonatal period, and the rats were analyzed at 6 months of age. He aupper panels show transversal section of the anterior commisure stained for myelin. The lower panels show electro microscopy analysis. The number of myelinated axons is reduced in the hypothyroid rats in parallel to an increased number of small diameter axons. Those axons reaching a critical size have near normal myelin content, but still present structural defects (FromBerbel P et al, Behav Brain Res. 64:9-14, 1994).

THYROID HORMONES IN THE BRAIN

The concentrations of T4 and T3 in the brain are controlled by very efficient regulatory mechanisms involving thyroidal secretion, transport to the brain, expression of deiodinases and, in the fetus,

transplacental passage of thyroxine (fig 4). T3 equilibrates rapidly between the plasma, liver, or kidney pools, whereas the brain pool equilibrates more slowly. Therefore, when T3 alone is administered as a constant infusion, to thyroidectomized rats, the liver and kidney require lower doses than the brain. However, when T4 is administered, brain T3 is normalized at doses that result in relatively low concentrations in plasma or liver (55, 56). In addition, the brain T3 concentration is maintained within a narrow range under a wide range of T4 dosage, thus avoiding T3 excess.



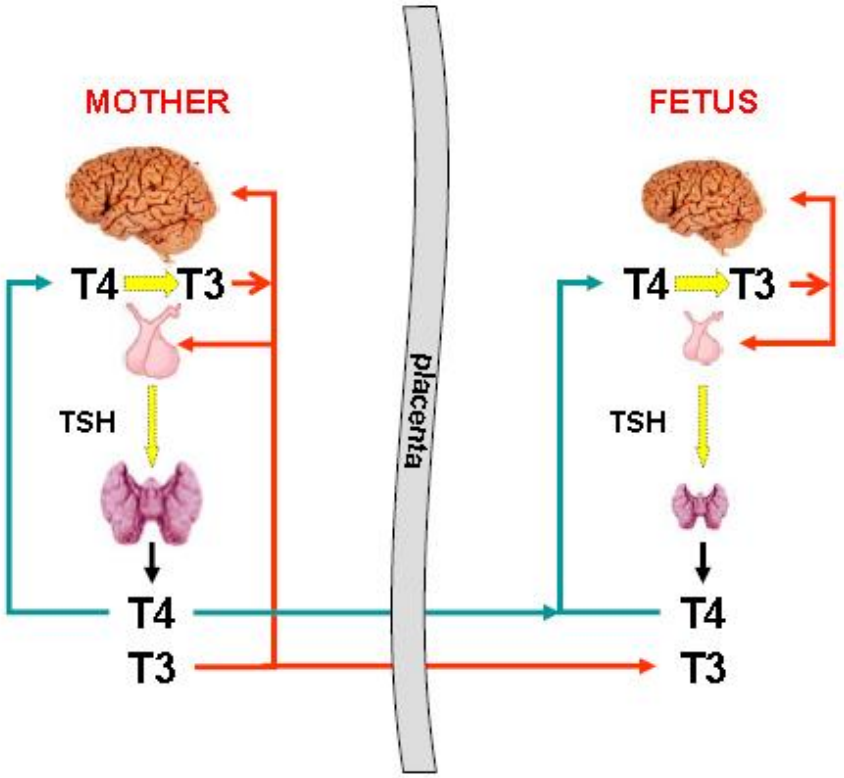


Fig 4.- The upper panel shows the T3 concentration in brain after administration of either T4 or T3 to the mother. Pregnant rats treated with MMI in order to block the maternal and fetal thyroids, were given either T4 or T3 as continuous infusion. T3 concentrations were measured in the brains of the mothers and the fetuses at term. In the mother, increasing doses of T3 led to a proportional accumulation of T3 in the brain. The normal T3 concentration was reached only by a narrow range of T3 doses. Administration of T4 led to a more flat accumulation of T3, and the normal T3 concentration was achieved through

a wide range of T4 doses. On the other hand, ddministration of T3 failed to increase T3 concentration in the fetal brain, whereas administration of T4 efficiently normalized fetal brain T3 concentrations (From Calvo R et al, J Clin Invest. 86:889-99, 1990. The cartoon in the lower panel offers an explanation for these results. In the mother, brain T3 derives partially from circulating T3 and from T4 deiodination. Circulating T4 and T3 in the fetus derive from the fetal thyroid gland and from the mother. However, brain T3 is exclusively a product of T4 deiodination.

When T4 is administered to pregnant dams previously treated with MMI in order to block the maternal and fetal thyroids, T3 is found in the fetal brain. In contrast, if T3 is given to the dams, it is not detected in the brain despite crossing the placenta and reaching other tissues such as the liver (57, 58). These results indicate that the T3 found in the fetal brain after T4 administration to the pregnant dams is generated locally, because for some reason circulating T3 in the fetus does not reach the brain.This is not so in postnatal and adult rodents in which brain T3 derives from the blood and from T4 deiodination. In the absence of D2 (see next section) brain T3 is about 50% of normal (59).

Sources of Thyroid Hormone for the Fetus

Before onset of function of its own thyroid gland, the only source of thyroid hormone for the fetus is the maternal thyroid gland. Thyroid hormones are present in the rat embryos as early as 3 days after implantation and in the fetus well before onset of fetal thyroid gland function (60-65). During fetal development the proportion of hormone in the fetus originating in the fetal gland increases, and that of maternal origin decreases, but in the rat at term maternal T4 still accounts for about 17.5% of the fetal extra thyroidal thyroxine pool (66). In humans T4 is already present in the coelomic fluid bathing the yolk sack, as early as the 6th gestational week (67). In the fetal brain, T4 and T3 are present in significant amounts by the 10th week after conception (68). At term, about 30-50% of T4 present in neonates represents the maternal contribution (69).

Expression and Regional Distribution of Deiodinases

The predominant deiodinases expressed in brain are D2 and D3, products of the Dio2 and Dio3 genes respectively (70). Deiodinases are membrane-anchored proteins. D1 and D3 are anchored to the plasma membrane with the catalytic site exposed to the intracellular compartments, whereas D2 is anchored to the endoplasmic reticulum, with the catalytic site exposed to the lumen (70, 71). D3 in the plasma membrane is oriented with its catalytic site exposed to the extracellular fluid (72). In this way it should have easy access to extracellular iodothyronines. There is also evidence that iodothyronines need to be internalized in the cell in order to act as D3 substrates (73). This could be due to the rapid internalization of D3 into endosomes. (72) As described below, Dio2 and Dio3 are expressed in different cells, Dio2 in astrocytes and Dio3 in neurons. Therefore, astrocytes generate the active T3, whereas neurons degrade T4 and T3 to rT3 and T2, respectively. Furthermore, D2 activity is regulated directly by T4, which increases D2 degradation, whereas Dio3 expression is increased by T3. In other cellular contexts the balance between D2 and D3 activities is also regulated by Sonic Hedgehog, which increases D2 degradation and induces Dio3 expression (74, 75). Whether Shh plays similar roles in the developing brain is not known.

Type 2 deiodinase

In Dio2-expressing tissues, such as brain, brown adipose tissue and pituitary, 50% or more of T3 derives from local T4 deiodination (76, 77). In the adult rat brain as much as 80% of nuclear bound T3 is formed locally from T4 (78). D2 activity is detectable in the rat fetal brain, markedly increasing by the end of pregnancy to adult levels (79, 80). At the same time, brain T3 increases by about 18-fold (80). D2 activity increases in hypothyroidism, and is very sensitive to the administration of T4 (81). In iodine deficiency the increased D2 activity tends to maintain T3 concentrations normal despite greatly reduced T4 concentrations in plasma and brain (82). The main regulation of D2 activity occurs at the post translational level involving the actin cytoskeleton (83-85) and the ubiquitin-proteasome pathway (86, 87). In addition, Dio2 expression is also regulated at the mRNA level (88-91).

Strongest Dio2 expression in the brain (Fig 5) occurs in the hypothalamus (92), in specialized glial cells known as tanycytes, which partially line the walls of the third ventricle (91-93). These cells extend long processes to the adjacent hypothalamus and the median eminence (94) ending in capillaries and axon terminals. The tanycytes are involved in the uptake of T4 from the capillaries of the median eminence and basal hypothalamus and/or from the CSF and its conversion to T3. T3 formed in tanycytes would be released back to the CSF and reach other brain regions by diffusion, or it may be delivered to hypothalamic nuclei directly from the tanycytes processes. It is likely that T3 reaches in this way the paraventricular nucleus (PVN) where T3 regulates TRH production and does not express D2 (93). T3 produced by tanycytes could also participate in anterior pituitary regulation after delivery to the portal vessels (95). Activation of the Dio2 gene in the basal hypothalamus is involved in photoperiods and seasonal breeding in birds and mammals (96, 97).

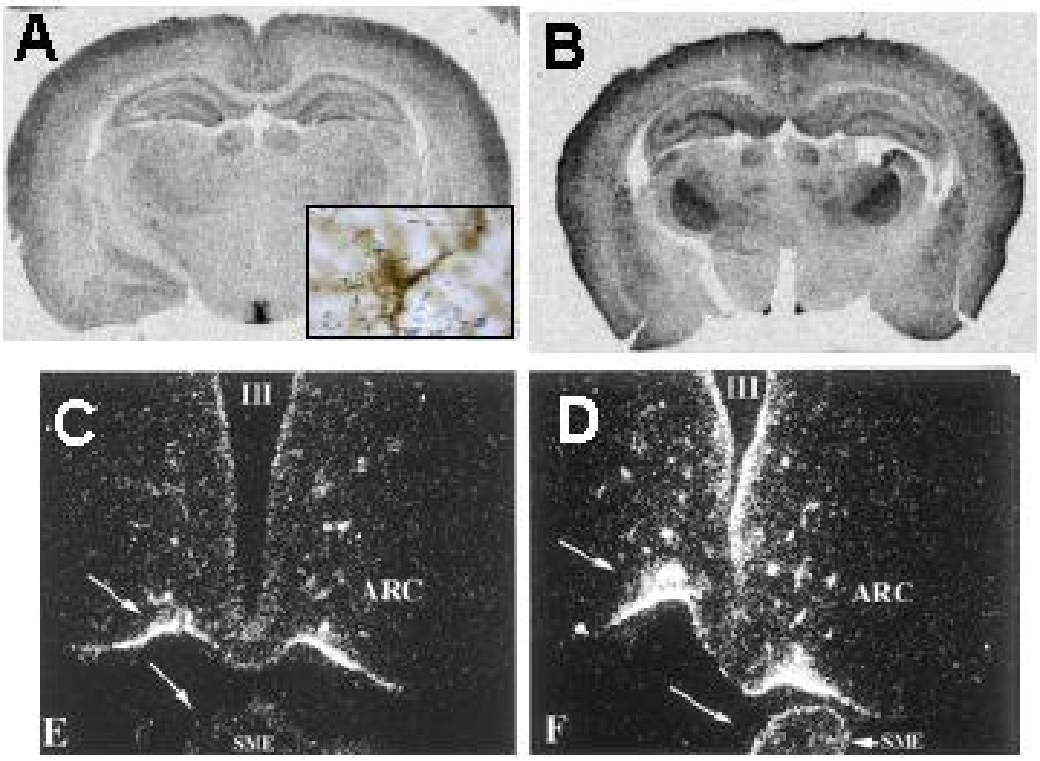


Fig 5.- Type 2 deiodinase mRNA expression in the rat brain. A: euthyroid rat. D2 mRNA is distributed all over the brain with high concentration in walls of the lower third of the 3rd

ventricle. In this region, D2 is expressed in specialized glial cells called tanycytes. In the rest of the brain D2 is expressed in the astrocytes (the inset shows D2 mRNA hybridization silver grains over a stained astrocyte). B: D2 expression in a hypothyroid rat. An increased expression is observed in most parts of the brain, but especially in the barrel cortex and some structures such as the ventromedial nucleus, serving as relay stations for somatosensory pathways. C and D: Dark field photomicropgraphs of the 3rd ventricle, infundibular region and median eminence showing high expression of D2 mRNA in this area (C), and its increased expression in iodine deficient rats. Upper panels are data from the author. Lower panels are adapted from Peeters et al, Am J Physiol Endocrinol Metab 281: E54-E61, 2001.

Dio2 is also expressed in astrocytes throughout the brain (90, 93). Specific astrocytic expression of the Dio2 gene has also been found in whole genomic analysis of purified brain cells, revealing that Dio2 is one of the top 50 astrocyte-specific genes (98). Localization of D2 in astrocytes and tanycytes has also been found in the human hypothalamus (99). Expression of D2 in astrocytes contrasts with the localization of the T3 receptor, which is mainly neuronal (100, 101). Therefore, the current thinking is that astrocytes are involved in the uptake of T4 from the brain capillaries, and in T3 generation and further delivery to neurons (see below). This is reminiscent of other forms of coupling between glia and neurons (102) in glucose metabolism, or in the generation of glutamate and GABA. Also in the cochlea, D2 is present in the connective tissue (103) whereas the T3 receptor is expressed in the sensory epithelium and spiral ganglion (103-105).

Dio2 knock out mice (59) have reduced T3 concentrations in brain, similar to those of hypothyroid mice; however, the mice have minimal neurological impairment. Tests of locomotion, memory, anxiety, etc are normal or reveal minimal impairment. In the earliest studies on Dio2 knock out mice it was realized that expression of some T3 responsive genes, such as Nrgn (RC3/Neurogranin) was not altered in the absence of D2, despite having similar brain T3 concentrations as the hypothyroid mice. The cause for this discrepancy is not known, but the possibility of different roles for the T3 generated locally from T4, and the T3 reaching the neurons directly from the blood, is very intriguing. In fact, we have recently found that in the absence of D2, when all brain T3 is from the circulation, expression of most positively regulated genes in the cerebral cortex is unaffected. In contrast, the negatively regulated genes show an expression level similar to hypothyroid animals. (106, 107). It may be that more T3 is needed intracellularly for gene repression than for gene induction, and that the extra T3 needed is provided by D2 activity. Double Dio1/Dio2 knock out mice also have normal blood and tissue concentrations of T3, similar to those of hypothyroid mice or Dio2 knock outs but Nrgn expression is reduced in the cortex. The double knock outs also have minimal neurological phenotype (108).

Type 3deiodinase

D3, the product of the Dio3 gene, inactivates thyroid hormones through inner ring deiodination (109). D3 activity is highest in placenta and in fetal tissues and decreases after birth (79, 110-112). In the human placenta, D3 activity is 200 times higher than that of D2 at all gestational ages (113, 114). Although in cultured astrocytes Dio3 expression can be induced by growth factors (115-117), in vivo it is expressed in neurons (118, 119).

D3 plays an important role in the control of T3 concentration in developing tissues. For example, in metamorphosis D3 is negatively correlated with responsiveness to thyroid hormone of tadpole tissues (120-123). In mammals placental D3 controls the transfer of maternal thyroid hormone to the fetus

(124). Dio3 expression is very high in the uterus at the implantation site and in the epithelial cells of the uterine lumen surrounding the fetal cavity (125). In the newborn rat brain discrete and intense expression of D3 occurs in neurons located in areas involved in sexual differentiation of the brain (118, 126), suggesting that these areas need to be protected from a possible interfering action of T3 during critical periods of sexual brain differentiation (127). D3-deficient mice (128, 129) have profound alterations in thyroid hormone economy, with greatly elevated thyroid hormone concentrations during the perinatal period. This hypermetabolic situation progresses into a state of central hypothyroidism, maintained through adulthood. In the brain, the expression of thyroid hormone target genes is elevated during the early postnatal period, and then decreases, in parallel to the thyroid state (130). D3 activation reduces the effects of high doses of T3 on gene expression, and therefore protects the brain from excessive T3 (107). The effect of T3 on the Dio3 gene is selectively mediated through the TRα1 receptor subtype (131).

Astrocyte-neuron coupling in the regulation of thyroid hormone action in the brain.

As indicated above, expression of Dio2 in astrocytes led to the hypothesis that in the brain T4 was converted to T3 in the astrocytes and delivered to neurons in response to local needs (93). This hypothesis was later refined by the realization that D3 was present in the neurons, thereby providing a control of neuronal T3 through degradation. It was later demonstrated in vitro using a co-culture assay that T3 produced in astrocytes could influence neuronal gene expression (132). Proof that this mechanism indeed operates in vivo was recently provided by our laboratory. First, we showed that circulating T3 needs the presence of the Mct8 transporter (see below) in the blood-brain barrier (BBB) to influence neuronal gene expression (133). Absence of Mct8 did not affect the action of T4, presumably because it crosses the BBB through another transporter (see below) and the T3 produced in the astrocytes would compensate the deficit of T3 from the circulation. That this was indeed the case was demonstrated by analyzing cerebral cortex gene expression in mice that lacked Mct8, D2, or both (106). While at least the positively regulated genes were normally expressed in the single Mct8 or D2 deficiency, the absence of both, Mct8 and D2, led to hypothyroid levels of gene expression. This experiment demonstrates the relevance of the two sources of T3, the circulation, and the astrocyte, for thyroid hormone action in the neurons.

Transport of Thyroid Hormones to the Brain

The brain barriers

The passage of substances from blood to brain is restricted by the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (blood-CSF) barrier (134) (Fig 6). The BBB is formed by the endothelial cells of brain capillaries which are apposed by tight junctions, so that substances must leave the circulation and enter the brain parenchyma through transcellular transport. Surrounding the brain capillaries are the astrocytic end-feet. It has been proposed that the astrocyte membranes do not cover completely the capillary surface, leaving patches of capillary wall in contact with the insterstitial fluid (135). Therefore T4 and T3 transported through the BBB could reach the astrocytes through their end-feet or be delivered directly to the interstitial fluid through the spaces between astrocytes. Other studies however show that the astrocytes cover completely the capillaries, leaving only narrow spaces for free diffusion (136).

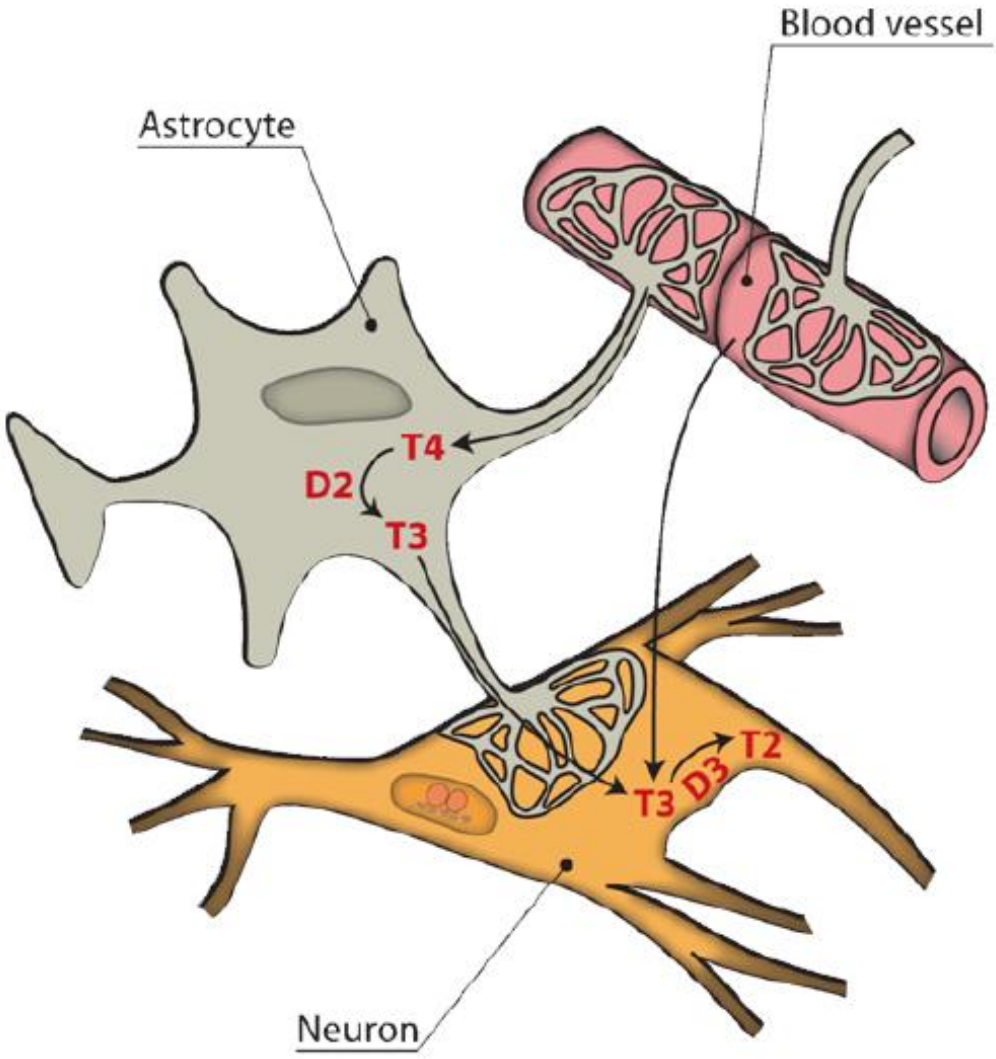


Fig 6.- Entry of thyroid hormone to the brain through the blood-brain barrier (BBB). T3 can reach the target neurons via two ways. One is from the astrocyte, after 5'deiodination of the T4 taken up from the blood through the astrocyte end-feet. T3 can also reach the neurons directly from the extracellular matrix after crossing the BBB. In the neurons, T3 is degraded to T2 by D3. The end-feet of the astrocytes are fenestrated, leaving part of the external capillary surface, and neuronal membrane in direct contact with the extracellular matrix.

The blood-CSF barrier is formed by the epithelial cells lining the ventricular side of the choroid plexus. Administration of labelled T4 or T3 leads to a rapid labelling of the choroid plexus and the appearance of the hormones in the CSF (137). Thyroid hormones are present in the CSF at less than ten times the concentration in serum (138-140), but the free hormone fraction is several fold higher in the CSF due to the low protein concentration. It was suggested that transthyretin (TTR), the major protein of the CSF in many species, plays a role in T4 transport in the choroid plexus (141, 142). However, the T4 transfer rate from plasma to tissue compartments, including the brain, is normal in transthyretin null-mutant mice (143, 144).

The bulk of hormone that reaches most brain structures does it through the capillaries in the parenchyma. The fraction of brain thyroid hormone that is transported through the choroid plexus and the CSF has been estimated to be around 20% (145). Labelled T4 injected directly into the CSF accumulates mainly in the median eminence (146, 147).

Thyroid hormone transporters

The transport of thyroid hormone through the plasma cell membrane is facilitated by several classes of membrane transporters (for recent reviews see (148-153)). The discovery that mutations in one of these transporters, the monocarboxylate transporter 8 (MCT8, SLC16A2 gene) cause a syndrome of severe neurological impairment and thyroid hormone abnormalities, provided a definitive proof for their physiological importance (154, 155) (see later section). The membrane transporters for thyroid hormones belong to several families including the Na+-dependent organic anion transporter (NTCP), the Na+-independent organic anion transporting polypeptides (OATP), the heterodimeric amino acid transporters (HAT) containing the light chains LAT-1 (SLC7A5) and LAT-2 (SLC7A8), and the monocarboxylate anion transporters (MCT) MCT8 and MCT10 (SLC16A10 gene). These transporters have a wide range of tissue distribution, with overlapping patterns of expression in most of them (156).

MCT8 and MCT10 are specific for the transport of iodothyronines, with slightly higher affinity for T3 than for T4. MCT10 have similar kinetic properties than MCT8, and may mediate both, influx and efflux of the hormones. Rodent Mct8 is strongly expressed in the choroid plexus. It is also expressed in the membrane of tanycytes and neurons. In addition, recent data have demonstrated anatomically and functionally that Mct8 expression in the BBB is very important to understand differential T4 and T3 transport through the BBB (133, 157). While MCT8 transports T4 and T3, OATP1C1 (SLCO1C1) transports preferentially T4. This transporter is also expressed in the endothelial cells of the capillaries forming the blood brain barrier, and in the choroid plexus (158). Based on the differential expression of transporters, and the comparative effects of T4 and T3 in the brain of Mct8 knock out mice (106), it can be proposed that in rodents T4 is transported by Oatp1c1 to the astrocytes where it is converted to T3. Circulating T3 crosses the BBB primarily through Mct8, probably reaching the brain interstitial fluid directly. Astrocyte-produced, or blood T3 may be transported to neurons primarily through Mct8 but may also do so through alternative transporters (133). Actually astrocytic-produced T3 reaches the neurons in vivo in the absence of Mct8 (106).

THYROID HORMONE RECEPTORS IN THE BRAIN

In rats the nuclear thyroid hormone receptor protein is present in brain at embryonic day 13.5-14 i.e., several days before onset of thyroid gland function (Fig 7). The receptor increases subsequently and reaches a maximum on postnatal day 6 (159-161). Total brain receptor occupancy by the hormone increases in parallel with plasma and cytosol total and free T3 with a maximum of 50-60% on postnatal day 15 (162). All receptor isoform mRNAs are expressed in the brain, but the predominant TR isoform is TRα1, widely distributed in the CNS from E14 to adulthood (100, 101, 163). From E19 to P0, TRα2 is present in the outer part of the cerebral cortex and hippocampal CA1 field. During the late fetal stage TRα1 becomes expressed in the piriform cortex, superior colliculus, and pyramidal layer of the hippocampus, and in the granular layer of dentate gyrus. In adult rats TRα1 expression is prominent in the cerebral cortex, cerebellum, hippocampus, striatum and olfactory bulb. The pattern of TRβ1 expression during development is different than that of TRα1, with restricted low expression during the fetal period, and increasing during the postnatal period and through adulthood. TRβ1 mRNA can be

detected at E15.5 in the upper tegmental neuroepithelium. Between E17 and E20 only low levels are present in the brain, especially in the pyramidal layer of the hippocampus. On P0 a drastic increase occurs in the accumbens, striatum, and hippocampus. From around P7 TR 1 becomes also expressed in the cerebral cortex. The patterns of expression of TRβ1 and TRα1 overlap (Fig 8), but in some cells, one of the isoforms is expressed preferentially. For example, in the cerebellum, differentiated granular cells express TRα1 while Purkinje cells express TRβ1. Recent studies by Vennström and coworkers (164) have shown that TRα1 is expressed essentially in all postmitotic neurons, with the notable exception of Purkinje cells, and in a few glial cell types.

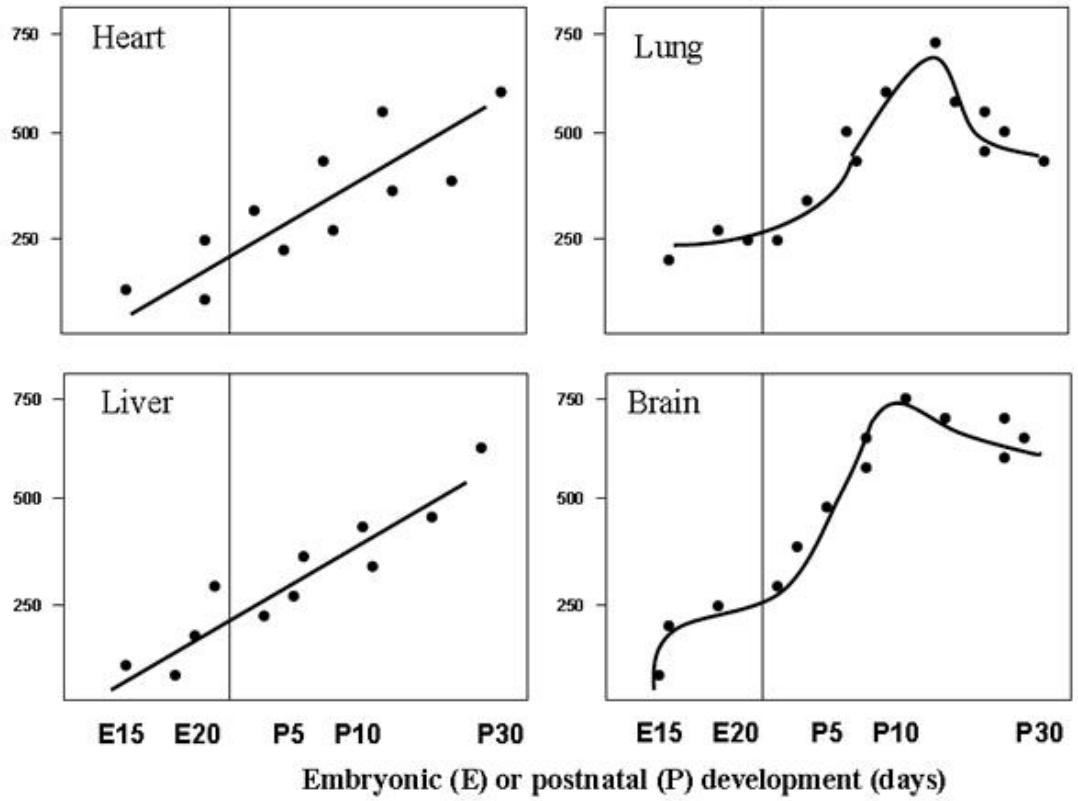


Fig 7.- Ontogeny of T3 receptors in rat organs. Receptor concentration was measured by T3 binding assays. From Perez-Castillo et al, Endocrinology 117:2457-2461,1985.

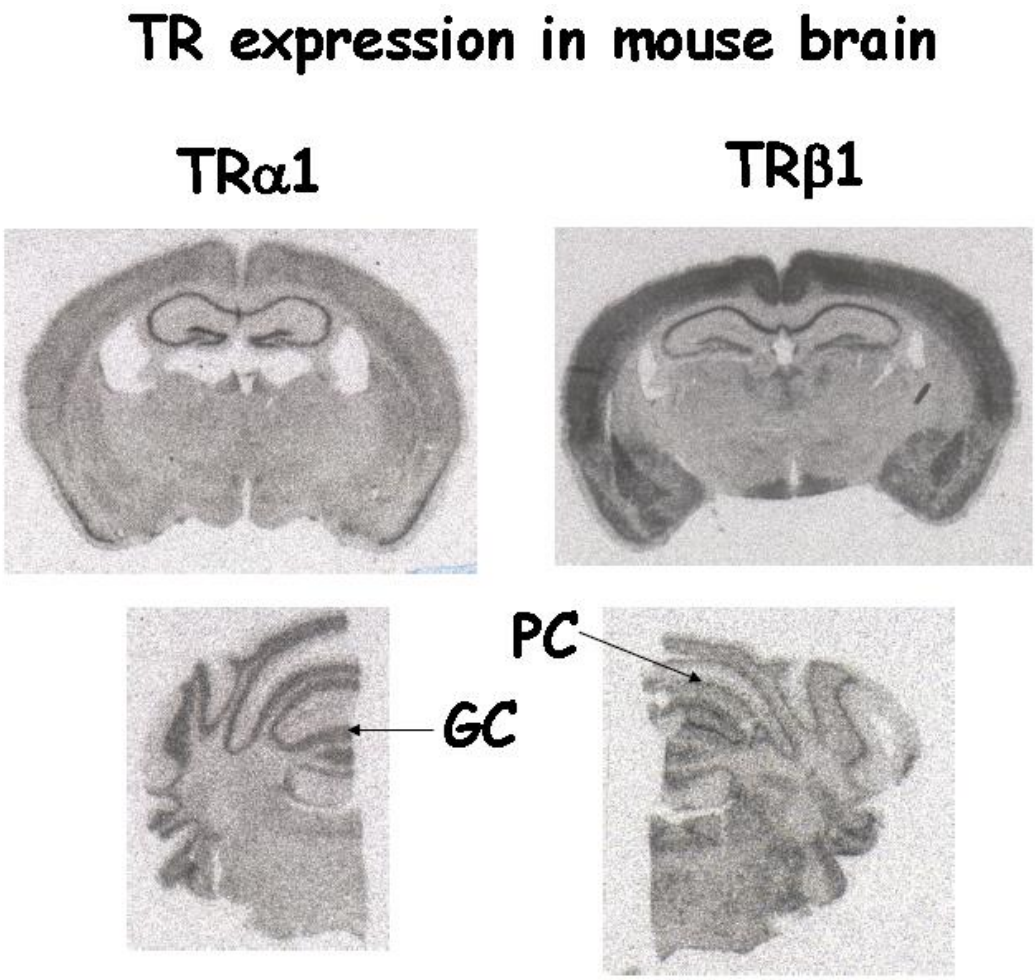


Fig 8.- T3 receptor mRNA expression in the mouse brain, by in situ hybridization with TRalpha1 and TRbeta1-specific probes. In the cerebrum (upper panels) there is an overlapping distribution of both receptor subtypes, with some differences in the hippocampus, amygdala ans hypothalamus. In the cerebellum (lower panel) TR□ 1 is expressed in the granular layer (GC), whereas TR□ 1 is expressed in the Purkinje cell layer (PC).

As with the mRNA, the predominant receptor protein in the brain is TR□ 1, which accounts for about 70-80% of all receptor subtypes. In addition to TR□ 1 and TR□ 1, TR□ 2 isoform, initially considered to be pituitary-specific (165), was later detected by in situ hybridization in several regions of the brain such as the rostral caudate, the hippocampus and the hypothalamus (100). Actually the TR□ 2 protein contributes by about 10 % to the total receptor protein in different tissues, including the brain (166). By immunohistochemistry the TR□ 2 protein was found widely distributed in the brain, in regions where the mRNA cannot be detected, such as layers II-VI of the cerebral cortex, and Purkinje cells of the cerebellum (167). Quantitative studies on the concentrations of TR mRNAs and proteins in different tissues were carried out by Ercan-Fang et al (table 1) (168).

Table 1: TR isoform protein and mRNA concentrations in rat tissues		
	Protein molecules/cell	mRNA molecules/cell
TR□1		
Pituitary	2310	0.128

Liver	963	1.11
Brain	2360	16.4
Kidney	626	2.50
Heart	1300	2.70
TR□1		
Pituitary	1470	0.873
Liver	3900	5.10
Brain	819	20.2
Kidney	578	13.5
Heart	1460	7.37
TR□2		
Pituitary	5240	0.631
Liver	829	<0.00689
Brain	328	<0.00684
Kidney	178	<0.00501
Heart	506	<0.00453

The three TR isoform proteins are present in all tissues. This includes the TR□2 isoform, despite that the corresponding mRNA is only detectable in pituitary. The concentrations of receptor protein were measured by specific immunoprecipitation of labelled T3 after incubation with tissue nuclear extract. The concentrations of mRNA were measured in northern blots using quantitative procedures. Data from (168).

In the human brain, the receptor protein is present at low levels in the fetus around the 10th week postconception (68). The T3 receptor mRNAs can be detected during the first trimester (169). Receptor concentration increases 10-fold from the 10th to the 16th-18th weeks postconception (Fig 9). During this time the brain gains in weight and DNA content by about 5 fold, so that the total brain T3 receptor content increases 500 times. This period coincides with that of the active neuroblast proliferation (170). Not only the receptor, but also the ligand T3 is also present in brain from the 10th week of gestation, at enough concentrations to results in about 25% occupancy of receptor (171). At the same time, in other organs such as liver and lung, even with higher receptor concentration, only T4 was present on 18th weeks. This indicates that during the second trimester of pregnancy, the main source of T3 to the fetal brain is local deiodination of T4 by D2. Kester et al (172) showed that T3 increases in the cerebral cortex during the second trimester, in parallel with increased D2 activity. In the cerebellum however a high activity of D3 keeps T3 concentrations low (Fig 10) during the same developmental ages. These data are especially relevant in the context of the pathogenesis of neurological cretinism and for the consequences of maternal hypothyroxinemia on fetal brain development, as discussed below.

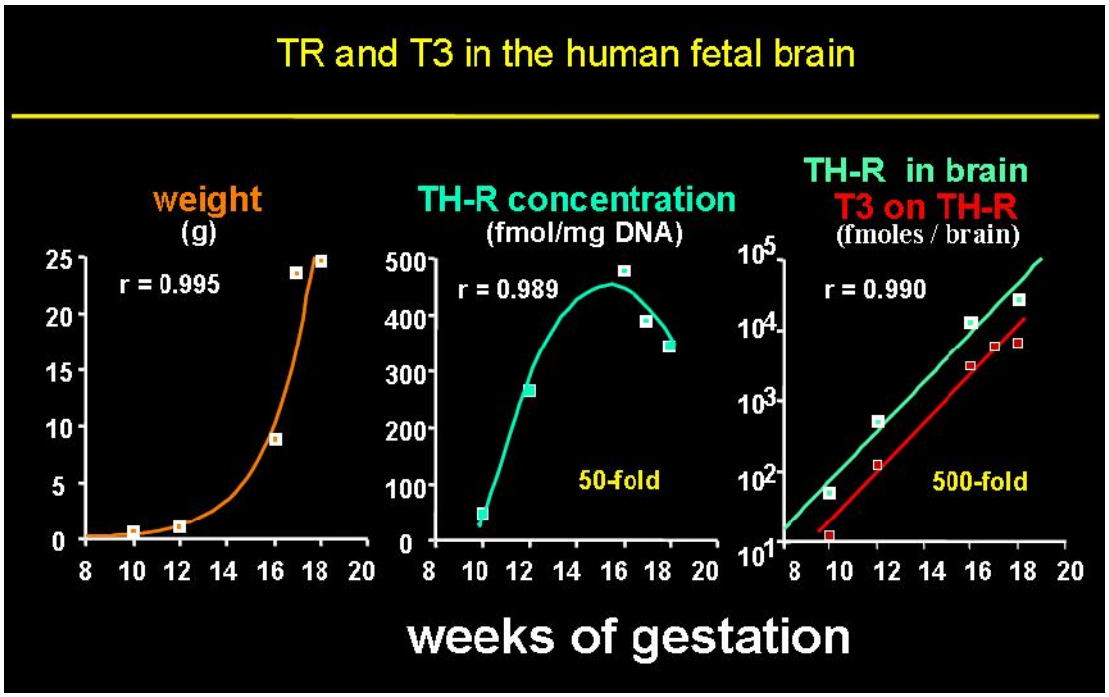


Fig 9.- Ontogeny of T3 receptor in the human fetal brain, measured by T3 binding assays. During the period analyzed the brain increases exponentially in weight, due mainly to the wave of neuronal proliferation (left panel). The receptor concentration increases 50-fold from the 10th to the 16th week (middle panel). In addition, despite the fact that the fetal thyroid glandis still not functioning, T3 increases in concentration during the same period, and can be found in the receptor fraction. Accumulation of T3 reflects the increasing activity of D2 in the cerebral cortex, as shown in fig 10. From Bernal and Pekonen, Endocrinology 114:677-679.

The distribution of T3 receptor mRNAs in vivo by in situ hybridization suggests a predominant neuronal expression. However, in neural cell cultures, T3 receptors have been detected in neurons, astrocytes and oligodendrocytes (173, 174). In dorsal root ganglia both sensory neurons and Schwann cells appear to express T3 receptors (175) but whereas neurons express permanently the receptor, glial cells do only transiently (176). Studies in vivo have shown that TR isoforms colocalize with oligodendrocyte markers but not with astrocytic markers (177) and other studies have demonstrated that in primary culture, rat astrocytes do not express T3 receptor, but only the TR 2 isoform (178, 179). As mentioned above, recent studies showed expression of the TR 1-encoding gene in only a few glial cell types (164).

MECHANISMS OF THYROID HORMONE ACTION IN THE BRAIN

Regulation of Brain Gene Expression by Thyroid Hormone

Most effects of thyroid hormones on developmental processes are carried out through the control of gene expression, but non-genomic actions have also been proposed (180). Many thyroid hormone-dependent genes have been identified mostly during the postnatal period. With few exceptions, the role of thyroid hormone is to accelerate the rate of gene expression changes during development. For example, myelin proteins accumulate rapidly in the brain during the first weeks of life in the rat, in parallel to an increased gene expression and RNA accumulation. The role of thyroid hormone is to

control the rate of gene transcription so that accumulation of the gene products occurs at the required rate. In the absence of thyroid hormone, mRNA and protein accumulation slows down. but their final concentrations in the tissue eventually attain a normal value (see an example of this phenomenon in figure 1). Another property of thyroid hormone regulation of gene expression in the brain is the regional specificity, even for genes regulated at the transcriptional level (181). The molecular basis for the time and regional specificity is mostly unknown. Transcription is the result of combinatorial activity of many transcription factors, and one of them is the thyroid hormone receptor, in genes regulated by thyroid hormones at the transcriptional levels. Therefore the action of thyroid hormone will depend upon the specific expression of the receptor and other transcription factors regulating the target gene in specific cells and developmental periods.

Many of the thyroid hormone-regulated genes identified during the postnatal period in the rat brain are sensitive to the hormone only during a time window that spans the first 2-3 weeks after birth (Fig 11). Most of these genes are not dependent of thyroid hormone in the fetal or in the adult brain. Based on these observations it was thought that the critical period of thyroid hormone sensitivity in the brain is limited to the first 2-3 postnatal weeks in the rat. However, this concept derives from the fact that most searches for thyroid hormone dependent genes in brain have been made during the postnatal period, at the peak of T3 receptor expression and occupancy. Although this period is probably the most sensitive to thyroid hormones in terms of the irreversible consequences of thyroid hormone deprivation, the rat brain is also sensitive, morphologically and functionally, and also in terms of control of gene expression in the fetus and in adult animals. Many genes have been identified regulated by thyroid hormone in brain, both by conventional Molecular Biology approaches, and by microarray analysis (106, 182-188). For clarity only a few selected examples will be described.

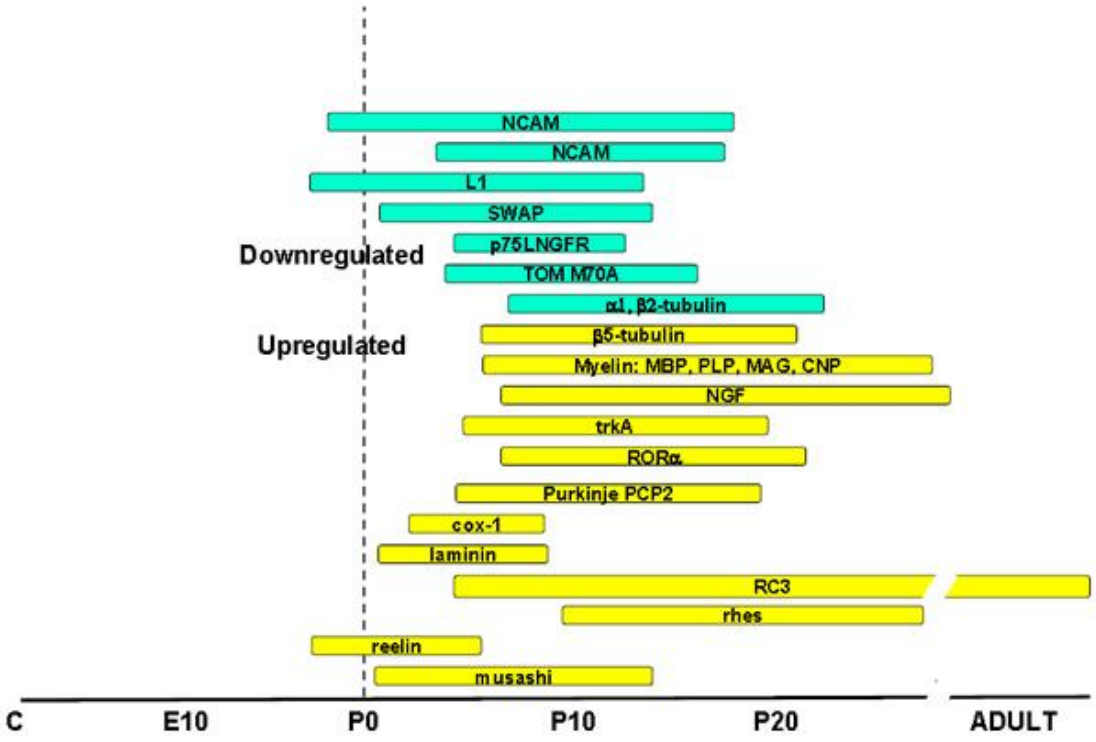


Fig 11: Regulation of brain gene expression by thyroid hormone during the postnatal period in the rat. The windows of sensitivity of regulated genes extend from the late fetal

period to the end of the first month, with some exceptions (RC3) extending to the adult. Only some selected examples are shown. The windows of sensitivity of the myelin genes are different for each brain region, so that in the more caudal regions (for example brain stem and cerebellum) the window is earlier than in the frontal regions (cortex).

The fetal period

Early efforts to demonstrate an effect of thyroid hormones on fetal brain gene expression led to the conclusion that the fetal rat brain at term is not sensitive to thyroid hormones (189). However, regulation of several genes, have been described in cultured fetal neurons (190) or in the brain in vivo (191-193). In the fetal cerebral cortex at the end of gestation thyroid hormone controls the expression of genes involved in biogenesis of the cytoskeleton, neuronal migration and growth, and branching of neurites. Interestingly, a large percentage of the thyroid hormone dependent genes were related to Camk4 signalling pathways (194). Camk4 was regulated directly by T3 in cultured primary neurons, confirming earlier results from other groups (195, 196)

The postnatal period

Most actions of thyroid hormone on brain development have been studied during the postnatal period in the rat (Fig 11). As mentioned above, the first 2-3 weeks of postnatal life is the period of maximal sensitivity of the rat brain to thyroid hormone, in parallel to the postnatal increase in thyroid hormone secretion, and brain D2 activity, and receptor and T3 concentrations. Important developmental events occur during this period: myelination, development of the cerebellum, dentate gyrus, and cochlea, glia cell proliferation, and terminal differentiation of neurons, with synapse formation and axon and dendrite sprouting. The corresponding period in the human is from about the 20th week of gestation to the first 2 years. Practically all these events are influenced by thyroid hormone, and genes involved in these processes whose expression is dependent on the thyroidal status have been identified.

The adult brain

In adult subjects thyroid hormones influence mood and behaviour, and thyroid dysfunction affects neurotransmitter systems (197) often leading to psychiatric disorders (198). High doses of T4 are effective in the treatment of bipolar depression (199, 200). In the adult rat striatum, administration of a large single T3 dose leads up-regulation of 149 genes and down-regulation of 88 genes (182). Physiological doses of T3 given for several days to hypothyroid animals led to up-regulation of 18 genes, and down-regulation of just one gene. Therefore, acute large doses of thyroid hormone causes large changes in gene expression, with more modest changes with lower doses. Some of the regulated genes are related to circadian rythms and to wakefulness, with one of them (Dbp or D-site binding protein) proposed as a candidate gene in bipolar disorders (201), and likely to be regulated directly by TRα1 (188). Many other genes were involved in striatal physiology as components of several signalling pathways (Fig 12).

Genes involved in Myelination

Neonatal hypothyroidism in the rat is associated with decreased myelin content, and reduced axon myelination. Thyroid hormone influences the expression of practically all myelin protein genes. The best characterized are those encoding the structural proteins (proteolipid protein (PLP), myelin basic protein

(MBP), and myelin associated glycoprotein (MAG) (202). The period of thyroid hormone sensitivity for these genes in the rat brain extends from about the end of the first postnatal week up to the end of the first month, but the timing of regulation has a strong regional component, in parallel with the wave of myelination (203, 204). Expression of myelin genes becomes normalized with time, even with continuous deprivation of thyroid hormones, but the number of myelinated axons remains lower than normal. The primary action of thyroid hormone on myelination is on oligodendrocyte differentiation (205). Thyroid hormone promotes differentiation of oligodendrocyte precursors in vitro (206). This effect is probably exerted through inhibition of the transcription factor E2F1 (207). The receptor involved in these actions of thyroid hormone is TRα1 (208, 209).

Mitochondria genes

An extensive review on thyroid hormone action on the mitochondria has been published (210). Mitochondria contain truncated forms of TR 1 and RXR , and thyroid hormone influences transcriptional activity of liver mitochondria (211, 212). In the brain, the thyroidal status influences mitochondrial morphology and function (213, 214) leading to changes in the expression of nuclear-encoded and mitochondrial-encoded mRNAs and proteins, such as 12S and 16S RNAs, cytochrome c oxidase subunits (214, 215), NADH dehydrogenase subunit 3 (216), and a protein import receptor (44).

Cell Migration

Thyroid hormone influences maturation of the radial glia, the path along which radial migration occurs in the cerebral cortex and the hippocampus (39). The mechanisms of thyroid hormone control of cellular migrations have not been defined in detail, but some of the molecules involved are regulated by thyroid hormone. Among them the extracellular protein Reelin, secreted by the Cajal-Retzius cells (46) of cortex and hippocampus, and by granular cells of the cerebellum, is under thyroid hormone control (217). The main function of Reelin is to stop migrating neurons, and it is essential for the inside-out pattern of cerebral cortex development. The protein Disabled1 (Dab1), a component of the Reelin signalling pathway is also under thyroid hormone regulation (46).

Thyroid hormones also regulate negatively the concentrations of other extracellular matrix proteins and adhesion molecules, of multiple functions including not only cell migration, but also neurite outgrowth, growth cone morphology, and axonal guidance and fasciculation. Among them Tenascin C, laminin, L1, and NCAM (181, 218-220). The concentrations of these proteins in neural tissue decrease during the postnatal period and thyroid hormones control the rate of this process.

Genes involved in Neural Cell Differentiation

Thyroid hormone controls the expression of many genes encoding proteins with roles on terminal cell differentiation, such as cell cycle regulators, cytoskeletal proteins, neurotrophins and neurotrophin receptors and extracellular matrix proteins. Among the cell cycle regulators, E2F1, p53, cyclins and cyclin-dependent kinase inhibitors are regulated by thyroid hormone in cell culture (221-223). As already mentioned E2F1 is involved in oligodendrocyte differentiation (207).

Neural cell shape is determined by the cytoskeleton, which consists of microtubules (Tubulin), microfilaments (Actin), and intermediate filaments, specific for neurons (Neurofilaments), glia (Glial Fibrillary Acidic Protein), or maturing cells (Vimentin, Nestin). Tubulins α1 and α2 are downregulated by thyroid hormone, and tubulinβ4 is upregulated (224, 225). Microtubule associated proteins (MAPs) are

also under thyroid hormone control at a posttranscriptional level. For example thyroid hormone regulates Map2 protein distribution in the Purkinje cell dendritic tree (226), and conversion of immature forms of Tau protein to mature forms of the TAU protein by alternative splicing of the Tau mRNA (227). The neurofilament genes Nefh, and Nefm are also under thyroid hormone control in the fetal and postnatal cerebral cortex (106, 194).

In addition to oligodendrocytes and radial glia, other glial cells are also influenced by thyroid hormones: astrocytes (228, 229) cerebellar Golgi epithelial cells (230), and microglia (231). As mentioned above, thyroid hormone influences the in vivo expression of astroglial genes such as those encoding Tenascin C, Laminin, and L1, which also have additional roles in neuronal migration and differentiation, and in axonal fasciculation. In vitro, the effect of T3 on astrocyte differentiation is blocked by □-adrenergic receptor antagonists (232).

Some of the effects of thyroid hormone on differentiation and survival might be mediated through control of neurotrophin expression. Interactions between thyroid hormone and NGF are important for the growth and maintenance of cholinergic neurons in the basal forebrain (18). Changes in NGF, TrkA and p75NTR after hypothyroidism have been described (233). In the cerebellum thyroid hormone also controls the expression of NT-3 in vivo and in cultured cerebellar granule cells and it was suggested that the control of Purkinje cell differentiation by thyroid hormone is mediated through NT-3 produced by granule cells (234).

Genes Involved in Cell Signalling

Proteins directly involved in intracellular signalling are also under direct control of thyroid hormone. Here are cited only a few examples to illustrate the diversity and pleiotropy of thyroid hormone effects on the brain. One of these proteins is RC3/Neurogranin (181), a protein kinase C substrate that binds Calmodulin in the non-phosphorylated state and in the presence of low Ca2+ concentrations (235). Neurogranin (Nrgn) is involved in synaptic plasticity (236) by regulating the free calmodulin available and its distribution within the dendritic spines. Nrgn knock out mice display alterations of spatial memory (237). Nrgn mRNA and protein are under thyroid hormone control in developing rats, mice and goats, and the effect is mediated at the level of transcription through a response element located in the first intron of the gene (238).

Rhes (Ras homolog enriched in striatum), or Rasd2 (239, 240) is striatal-enriched protein of the Ras family, with high homology with Dexras-1 (dexamethasone-inducible Ras protein) or Rasd1. These two proteins define a family within the Ras superfamily of small GTPases. Rhes regulates the AKT pathway, and mediates mTOR and dopamine signalling, and mutant-hungtintin toxicity, among other actions (241-243). The PCP-2 (Purkinje cell-specific protein 2, or L7) contains the G-protein regulatory motif GoLoco, involved in regulation of G□i protein signalling (244). Thyroid hormone also regulates in vivo and in cultured cells the expression of tubby, a gene expressed in hypothalamic nuclei encoding a protein that also acts through G-protein signalling (245, 246). Other genes involved in GPCR signalling are also under thyroid hormone control in the adult striatum (182).

Transcription Factors and Splicing Regulators

Regulation of proteins involved in transcription, and RNA stability and splicing is important for secondary influences in gene regulation. NGFI-A mRNA (Krox-24, Egr-1, Zif-268) is decreased in hypothyroid rats in several areas of the brain (247), and is induced by T3 at the promoter level in vivo

(248). T3 regulates the krüppel-like factor 9 (Klf9) transcription factor formerly known as BTEB (basic transcription element binding protein), a member of the Sp1 family of transcription factors, in vivo and in cultured neuronal cells (249, 250). Klf9 has been recently shown to mediate the actions of thyroid hormone in the loss of the regenerative capacity of Purkinje cells that occur during postnatal development (251). RORα, a member of the RZR/ROR family of orphan nuclear receptors is thyroid hormone dependent in the cerebellum. Disruption of the RORα gene is responsible leads to profound alterations in Purkinje cell growth and differentiation and granule cell migration (staggerer phenotype in mice) so that some of the actions of thyroid hormone on cerebellum could be mediated by this transcription factor (252). Also in the cerebellum, and other brain regions, the expression of the transcription factor Hairless is very sensitive to thyroid hormones (253). Hairless is a corepressor that heterodimerizes with the thyroid hormone receptor (254), so that in theory its induction would presumably tend to buffer other responses to thyroid hormone. Thyroid hormone also influences genes involved in RNA splicing, such as the mammalian homolog of the Drosophila splicing regulator Suppressor-of-white-apricot (SWAP) (255), and Musashi-1. Some of the posttranscriptional effects of thyroid hormone might be mediated through the control of RNA binding proteins controlling RNA stability.

Gene expression in the adult brain

In adult subjects thyroid hormones influence mood and behaviour, and thyroid dysfunction affects neurotransmitter systems [211] often leading to psychiatric disorders [212]. High doses of T4 are effective in the treatment of bipolar depression [213, 214]. In the adult rat striatum, administration of a large single T3 dose lead up-regulation of 149 genes and down-regulation of 88 genes [151]. Physiological doses of T3 given for several days to hypothyroid animals led to up-regulation of 18 genes, and down-regulation of just one gene. Therefore, acute large doses of thyroid hormone causes large changes in gene expression, with more modest changes with lower doses. Some of the regulated genes are related to circadian rhythms and to wakefulness, with one of them (Dbp or D-site binding protein) proposed as a candidate gene in bipolar disorders [215]. Many other genes were involved in striatal physiology as components of several signalling pathways (Fig 12).

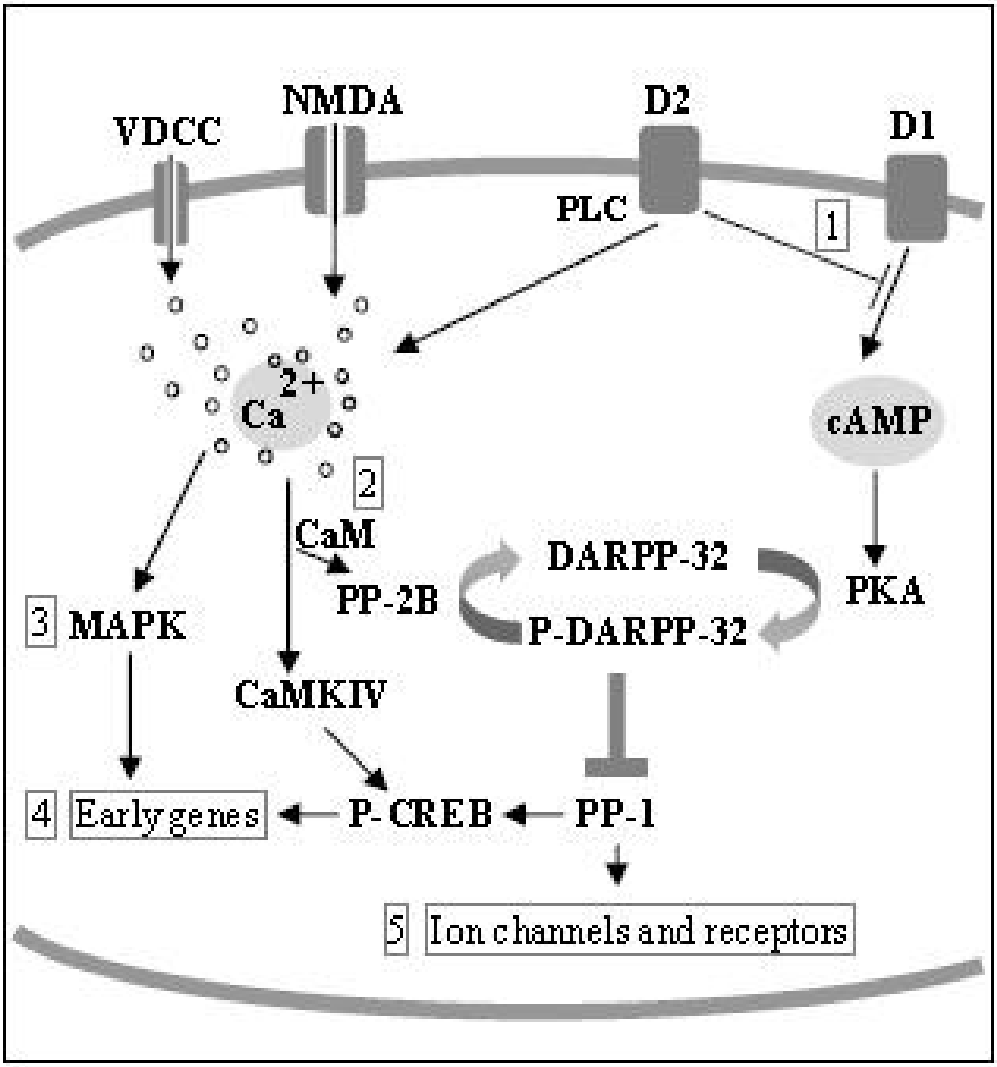


Fig 12: Regulation of gene expression by thyroid hormones in the adult striatum. Signaling pathways are schematically represented and the main groups of regulated genes shown in numerals. 1: G-protein coupled receptor signaling (Cnr1, Rgs9, Rasd2, Rasgrp1). 2: Ca2+/calmodulin pathway (Nrgn); MAPK pathways (Map2k3, Fos). 4: Early genes (Nr4a1, Arc, Dusp1, Egr1, Homer). 5: Ion channels (Scn4b). Abbreviations: VDCC, voltage dependent sodium channels, NMDA, N-methyl-D-aspartate, D1 and D2, dopamine receptors 1 and 2. From Diez et al, Endocrinology 149: 3989-4000, 2008.

Mechanisms of Gene Regulation by Thyroid Hormone

In some cases thyroid hormone responsive elements (TRE) have been identified in the promoter or intronic regions of thyroid hormone dependent brain genes. Among these, myelin basic protein (256), the Purkinje cell specific gene (PCP2) (257), the calmodulin binding and PKC substrate RC3 (238), prostaglandin D2 synthetase (258, 259) the transcription factor Hairless (253), the neuronal cell adhesion molecule (NCAM) (260) and the early response gene NGFI-A (248). Chromatin immunoprecipitation assays have identified many TRβ1 binding sites in the developing mouse

cerebellum (261) or cerebellar cells (188). Expression of other genes is regulated at the levels of mRNA stability (acetylcholinesterase), protein translation (MAP2) (226) or mRNA splicing (Tau, (227)). Regulation of splicing might be due to a primary action on the transcription of splicing regulators (262).

TRANSLATIONAL ASPECTS

The major causes of thyroid hormone deficiency during development are iodine deficiency, congenital disorders of the thyroid gland, and maternal and/or fetal hypothyroidism. In addition, the condition known as hypothyroxinemia, defined as low T4 in the presence of normal T3 and TSH, in the pregnant woman is suspected of a possible cause of developmental impairment. Less frequently, but of great importance are mutations of the thyroid hormone transporter MCT8. On the other hand, mutations of thyroid hormone receptors, THRA and THRB also cause variable degree of brain disturbances. The consequences of thyroid hormone deficiency on brain maturation greatly depend on the specific stages of development affected (Fig 13).

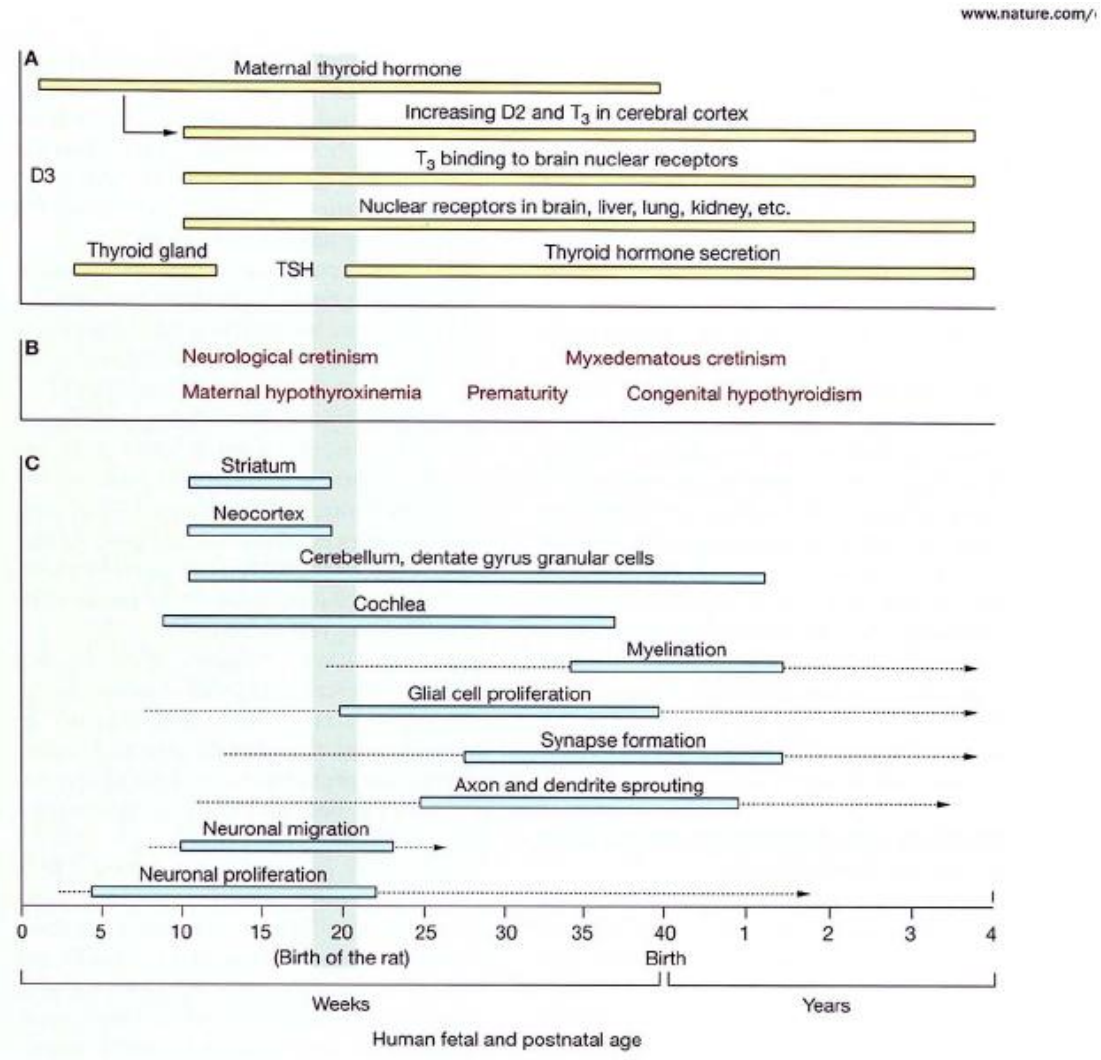


Fig 13: Relationship between human nervous system (C) and thyroid-related (A) developmental events, and the timing of insult of conditions due to thyroid hormone deficiency (B). For comparison with rat development, the equivalence for the birth of the

rat is shown by a vertical blue line around postmenstrual week 20. From Bernal, Nat Clin Pract Endocrinol Metab. 3:249-259, 2007.

Iodine Deficiency Disorders. Endemic Cretinism

Daily adult needs of iodine are of the order of 150, and 250 µg during pregnancy and lactation [222, 223]. Iodine deficiency causes a wide spectrum of abnormalities collectively known as iodine deficiency disorders [224], with high incidence of abortions and stillbirths, increased perinatal and infant mortalities, neonatal goiter and hypothyroidism, and various degrees of psychomotor and mental defects, and cretinism.

Iodine deficiency has been, and still continues to be, endemic in many regions of the planet. The consequence of severe iodine deficiency during development is the syndrome known as cretinism, of which two forms, Neurological and Mixedematous, were described by McCarrison [225] in the Himalayas and in Papua-New Guinea. In Neurological cretinism the thyroid gland is normal, and there are no signs of hypothyroidism. However, there is severe mental retardation, deaf mutism and a striato-pallidal disorder with spastic diplegia affecting the lower limbs [226]. On the other hand, mixedematous cretins [227] are hypothyroid, with short stature, poor sexual development, and craniofacial abnormalities. They are also mentally retarded but not as severely as neurological cretins, and signs of neurological involvement are observed only in a minority of cases. In affected regions, usually a combination of the two forms is mor frequently observed.

Until the 80's of last century, there were great controversies concerning the pathogenesis of each of these syndromes, and whether or not neurological cretinism was really due to thyroid hormone deficiency. Among other confusing reasons was the fact that early postnatal administration of iodine or of thyroid hormone was ineffective. It is clear today that the differences between the two forms of cretinism reflect different a different timing in the action, or lack of action, of thyroid hormones, according to developmental timing of expression of deiodinases, receptors, thyroid secretion, and maternal placental transfer of thyroid hormones. The clinical picture of neurological cretinism suggests damage to the striatum and the cerebral cortex during the second trimester of pregnancy, and it is due to the profound maternal hypothyroxinemia caused by severe iodine deficiency, during the first half of pregnancy. As described before (Fig 9), the T3 receptor is expressed from the second trimester, at a time when most T3 in fetal brain areas, such as the cortex depend on maternal T4, and local D2 activity (Fig 10). In contrast, Mixedematous cretinism is clearly due to failure of the fetal, and infant thyroid gland during the last trimester of pregnancy and the postnatal period. In many cases destruction of the fetal gland is due to a combination of iodine deficiency and dietary goitrogens.

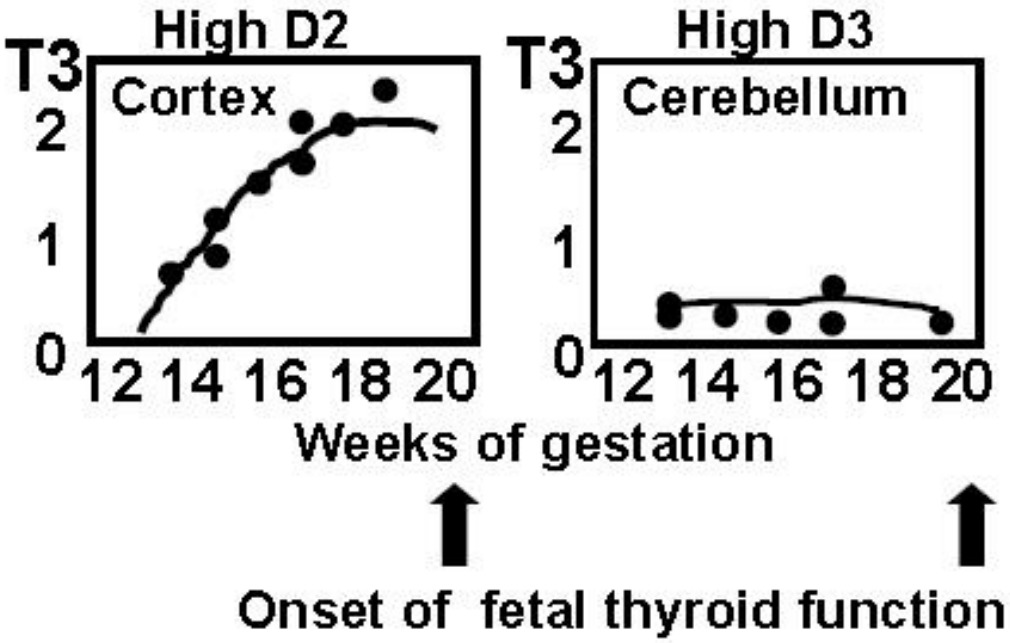


Figure 10: Concentrations of T3 in the fetal cerebral cortex and in the cerebellum. The cortex has high D2 activity, whereas the cerebellum has low D2 and high D3 activity. Accordingly, the coencentrations of T3 rise in the cerebral cortex and rfemain low in the cerebellum, reflecting different timing of T3 action. (From Kester et al, J Clin Endocrinol Metab. 89:3117-28, 2004).

Congenital Hypothyroidism

Congenital failure of the thyroid gland occurs in about 1 in 3,000-4,000 newborns (for a review see (269). Neonatal screening and early thyroid hormone treatment have efficiently prevented its consequences. Causes for Congenital hypothyroidism are thyroid dysgenesis including ectopic thyroid gland and agenesia, and inborn errors of thyroid biosynthesis. In a small percentage of thyroid dysgenesis mutations of genes involved in thyroid embryogenesis have been described (270). The great majority of cases are nonetheless due to non Mendelian mechanisms, with the involvement of several genes (271). An isolated family with truncated TSH receptor has also been described (272).

Despite the systematic and early diagnosis and treatment (273) of Congenital hypothyroidism in the prevention of mental retardation some affected individuals may remain with minimal brain damage, indicated by learning disabilities and disturbance of fine motor coordination. The success of thyroid hormone treatment depends on the severity, onset, and duration of hypothyroidism. Children at risk of having neurological sequelae even with early treatment may require a substantially higher replacement dose of T4 than moderate cases (274). It is therefore important to identify these children by the estimation of bone maturation and plasma T4 levels at diagnosis.

Maternal Hypothyroidism and Maternal Hypothyroxinemia

Maternal thyroid hormones cross the placenta (275), and up to 50% of fetal blood T4 at term is of maternal origin (69). Therefore, in congenital hypothyroidism maternal hormones protect the fetal brain until birth, and even children with total thyroid agenesia do not suffer from severe neurological deficits

as in neurological cretinism. The important role of maternal hormones is demonstrated in cases of severe maternal and fetal hypothyroidism. For example, in reported cases Pit-1 deficiency (276) or high titers of thyroid stimulation blocking antibodies (277, 278) circulating thyroid hormones are very low in the mother and in the infant. The children suffer from permanent sensorioneural deafness and irreversible neuromotor development resembling neurological cretinism.

Maternal hypothyroidism, in the presence of a normal fetal thyroid, is also harmful for fetal brain development (279, 280). High TSH in mothers during the second trimester of pregnancy is associated with a reduction of 4 points in the children IQ, and impaired attention, language, and visual-motor performance at 7-9 years. (280). Japanese children born from hypothyroid mothers who where treated after the 6th-16th week of gestation showed no signs of altered neurodevelopment (281). The authors argued against a major role of maternal T4 during early pregnancy. It is possible that iodine supply to the fetal thyroid is an important factor, considering the high iodine intake in Japan. At least in the rat, a good functioning of the the fetal thyroid gland is essential for cerebral cortex gene expression (58, 194). The need for antenatal thyroid screening of pregnant women to prevent impaired cognitive development of children born from women with low thyroid hormone levels was examined by Lazarus et al (282) It was found that the cognitive development at 3 years of age of children born from mothers with low thyroid hormone and/or high thyroid-stimulating hormone (TSH), with or without T4 treatment, was not different from that of the control group.

But there is also evidence that not only frank hypothyroidism, but maternal hypothyroxinemia is associated with a higher incidence of neurological alterations in children (283, 284), and increased incidence of Attention Deficit Hyperactivity Disorder (285). While the incidence of hypothyroidism in pregnant women is around 2.5%, hypothyroxinemia is much more prevalent, up to 30% (275, 286, 287), and it is usually due to mild iodine deficiency (288). In these situations, peripheral euthyroidism, and normal TSH levels, are maintained by several compensatory mechanisms, including preferential T3 secretion. For these reasons Iodine supplementation of women considering conception and during pregnancy and lactation is warranted (263). In rats Berbel et al have clearly shown that maternal T4 is needed for proper brain development (289). In pregnant women mild hypothyroxinemia due to iodine deficiency leads to altered neurocognitive performance of the progeny (290) but not if the hypothyroxinemia was corrected with iodine supplements during the first trimester.

Prematurity

Premature babies often present transient hypothyroxinemia lasting several weeks (291, 292), in about 85% of cases. This is due interruption of maternal thyroid hormone supply, and immaturity of the fetal thyroid axis. The issue of whether the hypothyroxinemia of prematurity is “physiological”, or deserves thyroid hormone treatment has been thoroughly discussed. It is clear that circulating total T4 and FT4 concentrations are lower in preterm neonates than in fetuses of comparable age still in utero (293-295). Therefore, the hypothyroxinemia of prematurity may have clinical consequences (296) with increased risk of neurological impairment (297), risk of cerebral palsy (298) and white matter damage (299). In an experimental model of prematurity, Berbel et al (289) have shown that deprivation of maternal hormones similar to that suffered by premature babies leads to altered development of the neocortex and hippocampus, and that the defects were corrected by T4 treatment of the dams. Despite the importance of this topic, the issue of whether preterm babies should be treated with thyroid hormone is not settled (300-302).

Thyroid hormone transporter mutations

Among the thyroid hormone transporters in the cell membrane, mutations have only been identified in MCT8, a 12 transmembrane protein encoded by SLC16A2, a gene located in chromosome X. They were first described by the groups of Refetoff (154) and De Visser (155) in children. Other cases were subsequently reported (303-305). Affected patients suffer from X-linked mental retardation, with severe developmental delay and neurological damage in addition to altered thyroid hormone levels in blood. Total and free T4 and rT3 are low, whereas total and free T3 are elevated. TSH is either normal or moderately elevated. The neurological syndrome consists of global developmental delay, lack of language development, profound mental deficiency, rotary nistagmus, impaired gaze and hearing, dystonic movements and severe proximal hypotonia with poor head control progressively developing into spastic quadriplegia. Some patients present paroxysmal dyskinesias (306). It was also recognized that the Allan-Herndon-Dudley syndrome (OMIM 300523) is due to MCT8 mutations (307). The neurological syndrome is likely due to defective T3 transport to neural cells during critical periods of fetal development (148, 308, 309), but the lesion responsible for the clinical phenotype is not known. The most likely possibility is hypomyelination . Actually the syndrome may be included within the leukoencephalopaties. About 5% of patients diagnosed of Pelizeaus-Merchbacher disease, a leukoencephalopaty usually due to PLP1 mutations, have instead MCT8 mutations (310).

Mct8-deficient mice (Mct8-/y) (311, 312) have also, as expected, elevated circulating T3 and reduced T4 and rT3. However they have minimal, if any, brain impairment (313), with only modest reductions in the expression of T3 target genes (133). The knock out mice are only partial models of the disease, and have been useful to dissect the mechanisms leading to the unusual pattern of thyroid hormone concentrations in blood (314). Restricted access of T3 to neurons, where Dio3 is expressed, impairs T3 degradation. The increased availability of T3 increases D1 activity in liver causing and increased rT3 degradation and T4 to T3 conversion, thereby reducing rT3 and T4 levels, and further contributing to increase T3. Additionally, thyroid gland secretion is altered because Mct8 is present in the basal membrane of the thyrocytes and plays an important role in thyroid hormone secretion (315, 316). In the brain there is a reduction of T4 supply to the astrocytes which causes an increased D2 activity and increased production of T3 from T4. Therefore, there are two important features of this syndrome: one is the concomitant increase in the activity of the two deiodinases, D1 and D2. The second is the coexistence of “hyperthyroid” (liver) and “hypothyroid” (brain) tissues. Even within the brain, the presence of alternative transporters in some cells might perhaps expose these cells to excessive T3, although this remains as a possibility (313).

There is strong evidence that the main restriction to the entry of T3 to neurons is not at the level of the neuronal membranes, but at the BBB (133, 157). Mct8, and other transporters, such as Oatp1c1 (317) are present in the membrane of capillary endothelial cells . In the absence of Mct8, T4 is still transported due to its higher affinity for Oatp1c1, therefore contributing, through T4 to T3 production, to compensate the lack of T3 transport through the BBB. It has been postulated that reason why Mct8 knock out mice do not show neurological impairment in contrast to patients, is due to compensation by a T4 transporter not be present in humans (157, 313). Indeed, the adult and juvenile primate BBB contains MCT8 but little OATP1C1 (318). The OATP1 transporter is detected immunohistochemically in the human fetal brain capillaries (157) but in lower amounts than in rodents.

T3 receptor mutations

The majority of cases (85%) of the classical form of Resistance to Thyroid Hormone (RTH) are due to mutations in the THRB gene (319). RTH patients may have learning disabilities, reduced IQ, and increased incidence of Attention Deficit and Hyperactivity Disorder. Thrb knock out mice, or knock in mice expressing receptor mutations are good models of RTH (320). In addition, the phenotypic analysis of these mice has revealed important roles of TRβ in maturation of somatosensory systems. Thrb mutant mice have deafness and impaired color vision, due to altered maturation of cochlear hair cells, and retinal photoreceptors (321, 322). Mice expressing a mutant TRβ1 with strong dominant negative property had cerebellar abnormalities reminiscent of severe hypothyroidism, and neuromotor disability (323).

THRA mutations have recently been described in humans (324, 325). The TRα1 mutant proteins display strong dominant negative activity. The patients suffer from growth retardation, delayed bone development, severe constipation, and mild cognitive deficits, with minimal alterations of circulating thyroid hormones: normal TSH, low T4/T3 ratios and strongly reduced rT3. T4 may be slightly reduced T4 or in the lower limit of normal range, and T3 may be slightly increased or in the upper limit of normal range. These alterations may be caused at least in part by deficient regulation of Dio3 which is a TRα1-regulated gene (131).

Previously, extensive work was aimed at defining the phenotypes of mice with TRα1 inactivation or harboring a mutant TRα1 with the thought that it could help to identify patients (326). In agreement with this, expression of mutant TRα1 with dominant negative activity causes retarded growth (327), low cerebral glucose consumption (328), retarded brain development, and neuromotor impairment in developing animals (329-331), and a profound anxiety in adult animals, ameliorated by T3 treatment (330). The consequences of TRα1 mutations may also be heterogeneous depending on the mutation (332-334).

An important observation was that absence of TRα1 in mice was not equivalent to mutations of the same gene. Despite the fact that most actions of thyroid hormone in brain are mediated by TRα1, absence of this receptor does not lead to a “hypothyroid” brain. In other terms, receptor deletion is not equivalent to hormone deprivation. This is due to the intrinsic transcriptional activity of the unliganded receptor. In the absence of the hormone, as in hypothyroidism, the unliganded receptor (or aporeceptor) has hormone-independent activity, either repression or activation of transcription, leading to a disturbance of processes which are normally controlled by thyroid hormones (335). Therefore, to some extent it can be said that the hypothyroid phenotype is the consequence of unliganded receptor activity. In the absence of receptor, this activity is suppressed and hypothyroidism is not as detrimental as in its presence (336, 337).

Since unliganded receptors have transcriptional activity, the question is if they have a have a physiological and developmental role in the absence of the hormone (335, 338). The receptors are expressed somewhat before the onset of thyroid gland secretion, and therefore occupancy of receptors during development is dependent as explained above, from the maternal hormones and the activity of deiodinases. D2 activity increases in the human fetal cerebral cortex during the second trimester, and T3 concentration increases in parallel (172). During the same stages, the cerebellum expresses mainly D3 and T3 is concentration is kept very low. These findings suggest that at the same time of development, the liganded and unliganded receptors are involved in development of the cerebral cortex and the cerebellum respectively. Evidence for a role of unliganded receptors have been demonstrated in studies on amphibian metamorposis (339), or in the development of the inner ear in mice (340).

1. Joffe RT, Sokolov STH. Thyroid hormones, the brain, and affective disorders. Crit. Rev. Neurobiol. 1994; 8: 45-63.
2. Laureno R. Neurologic manifestations of thyroid disease. The Endocrinologist 1996; 6: 467-473.
3. Ganguli M, Burmeister LA, Seaberg EC, Belle S, DeKosky ST. Association between dementia and elevated TSH: a community-based study. Biol. Psychiatr. 1996; 40: 714-725.
4. Anderson GW, Schoonover CM, Jones SA. Control of thyroid hormone action in the developing rat brain. Thyroid 2003; 13: 1039-1056.
5. Bernal J. Thyroid hormones and brain development. Vitam Horm 2005; 71: 95-122.
6. Bernal J. Thyroid hormone receptors in brain development and function. Nat Clin Pract Endocrinol Metab 2007; 3: 249-259.
7. Koibuchi N, Iwasaki T. Regulation of brain development by thyroid hormone and its modulation by environmental chemicals. Endocr J 2006; 53: 295-303.
8. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. Eur J Endocrinol 2004; 151 Suppl 3: U25-37.
9. Fisher DA, Polk DH. Maturation of thyroid hormone actions. In Delange F, Fisher DA, Glinoer D (eds): Research in congenital hypothyroidism, Edition New York: Plenum Press 1988; 61-77.
10. Holt AB, Renfree MB, Chek DB. Comparative aspects of brain growth: a critical evaluation of mammalian species used in brain growth research with emphasis on the Tammar wallaby. In Hetzel BS, Smith RM (eds): Fetal brain disorders. Recent approaches to the problem of mental deficiency., Edition Amsterdam: Elsevier/North Holland 1981; 17-43.
11. Morreale de Escobar G, Escobar del Rey F, Ruiz-Marcos A. Thyroid hormone and the developing brain. In Dussault JH, Walker P (eds): Congenital Hypothyroidism, Edition New York: Marcel Decker, Inc. 1983; 85-126.
12. Legrand J. Effects of thyroid hormones on Central Nervous System. In Yanai J (ed) Neurobehavioral Teratology, Edition Amsterdam: Elsevier Science Publishers 1984; 331-363.
13. Eayrs JT, Taylor SH. The effect of thyroid deficiency induced by methylthiouracil on the maturation of the central nervous system. J. Anat. 1951; 85: 350-358.
14. Madeira MD, Paula-Barbosa M, Cadete-Leite A, Tavares MA. Unbiased estimate of cerebellar granule cell numbers in hypothyroid and in sex-age-matched control rats. J. Hirnforsch. 1988; 29: 587-594.
15. Madeira MD, Paula-Barbosa M, Cadete-Leite A, Tavares MA. Unbiased estimate of hippocampal granule cell numbers in hypothyroid and in sex-age-matched control rats. J. Hirnforsch. 1988; 29: 643-650.
16. Manzano J, Cuadrado M, Morte B, Bernal J. Influence of thyroid hormone and thyroid hormone receptors in the generation of cerebellar GABAergic interneurons from precursor cells. Endocrinology 2007.
17. Gilbert ME, Sui L, Walker MJ, Anderson W, Thomas S, Smoller SN, Schon JP, Phani S, Goodman JH. Thyroid hormone insufficiency during brain development reduces parvalbumin immunoreactivity and inhibitory function in the hippocampus. Endocrinology 2007; 148: 92-102.
18. Gould E, Butcher LL. Developing cholinergic basal forebrain neurons are sensitive to thyroid hormone. J. Neurosci. 1989; 9: 3347-3358.
19. Gravel C, Sasseville R, Hawkes R. Maturation of the corpus callosum of the rat: II. Influence of thyroid hormones on the number and maturation of axons. J. Comp. Neurol. 1990; 291: 147-161.
20. Legrand J. Analyse de l'action morphogénétic des hormones thyroïdiennes sur le cervelet du jeune rat. Arch. Anat. Microsc. Morphol. Exp. 1967; 56: 205-244.

21. Dusart I, Flamant F. Profound morphological and functional changes of rodent Purkinje cells between the first and the second postnatal weeks: a metamorphosis? Front Neuroanat 2012; 6: 11.
22. Ruiz-Marcos A, Sanchez-Toscano F, Obregón MJ, Escobar del Rey F, Morreale de Escobar G. Reversible morphological alterations of cortical neurons in juvenile and adult hypothyroidism in the rat. Brain Res. 1980; 185: 91-102.
23. Ruiz-Marcos A, Sanchez-Toscano F, Obregón MJ, Escobar del Rey F, Morreale de Escobar G. Thyroxine treatment and recovery of hypothyroidism-induced pyramidal cell damage. Brain Res. 1982; 239: 559-574.
24. Gould E, Westlinddanielsson A, Frankfurt M, Mcewen BS. Sex Differences and Thyroid Hormone Sensitivity of Hippocampal Pyramidal Cells. J. Neurosci. 1990; 10: 996-1003.
25. Ruiz-Marcos A, Cartagena P, García A, Escobar del Rey F, Morreale de Escobar G. Rapid effects of adult-onset hypothyroidism on dendritic spines of pyramidal cells of the rat cerebral cortex. Exp. Brain Res. 1988; 73: 583-588.
26. Chen C, Zhou Z, Zhong M, Zhang Y, Li M, Zhang L, Qu M, Yang J, Wang Y, Yu Z. Thyroid hormone promotes neuronal differentiation of embryonic neural stem cells by inhibiting STAT3 signaling through TRalpha1. Stem Cells Dev 2012; 21: 2667-2681.
27. Mohan V, Sinha RA, Pathak A, Rastogi L, Kumar P, Pal A, Godbole MM. Maternal thyroid hormone deficiency affects the fetal neocorticogenesis by reducing the proliferating pool, rate of neurogenesis and indirect neurogenesis. Exp Neurol 2012; 237: 477-488.
28. Denver RJ, Hu F, Scanlan TS, Furlow JD. Thyroid hormone receptor subtype specificity for hormone-dependent neurogenesis in Xenopus laevis. Dev Biol 2009; 326: 155-168.
29. Ambrogini P, Cuppini R, Ferri P, Mancini C, Ciaroni S, Voci A, Gerdoni E, Gallo G. Thyroid hormones affect neurogenesis in the dentate gyrus of adult rat. Neuroendocrinology 2005; 81: 244-253.
30. Desouza LA, Ladiwala U, Daniel SM, Agashe S, Vaidya RA, Vaidya VA. Thyroid hormone regulates hippocampal neurogenesis in the adult rat brain. Mol Cell Neurosci 2005; 29: 414-426.
31. Lemkine GF, Raj A, Alfama G, Turque N, Hassani Z, Alegria-Prevot O, Samarut J, Levi G, Demeneix BA. Adult neural stem cell cycling in vivo requires thyroid hormone and its alpha receptor. Faseb J 2005; 19: 863-865.
32. Montero-Pedrazuela A, Venero C, Lavado-Autric R, Fernandez-Lamo I, Garcia-Verdugo JM, Bernal J, Guadano-Ferraz A. Modulation of adult hippocampal neurogenesis by thyroid hormones: implications in depressive-like behavior. Mol Psychiatry 2006; 11: 361-371.
33. Kapoor R, Desouza LA, Nanavaty IN, Kernie SG, Vaidya VA. Thyroid hormone accelerates the differentiation of adult hippocampal progenitors. J Neuroendocrinol 2012; 24: 1259-1271.
34. Lopez-Juarez A, Remaud S, Hassani Z, Jolivet P, Pierre Simons J, Sontag T, Yoshikawa K, Price J, Morvan-Dubois G, Demeneix BA. Thyroid hormone signaling acts as a neurogenic switch by repressing Sox2 in the adult neural stem cell niche. Cell Stem Cell 2012; 10: 531-543.
35. Kapoor R, Ghosh H, Nordstrom K, Vennstrom B, Vaidya VA. Loss of thyroid hormone receptor beta is associated with increased progenitor proliferation and NeuroD positive cell number in the adult hippocampus. Neurosci Lett 2011; 487: 199-203.
36. Hatten ME. Riding the glial monorail: a common mechanism for glial-guided neuronal migration in different regions of the developing mammalian brain. Trends Neurosci. 1990; 13: 179-184.
37. Rakic P. Mode of cell migration to the superficial layers of fetal monkey neocortex. J. Comp. Neurol. 1972; 145: 61-83.
38. Gaiano N, Nye JS, Fishell G. Radial glial identity is promoted by Notch1 signaling in the murine forebrain. Neuron 2000; 26: 395-404.

39. Martinez-Galan JR, Pedraza P, Santacana M, Escobar del Ray F, Morreale de Escobar G, Ruiz-Marcos A. Early effects of iodine deficiency on radial glial cells of the hippocampus of the rat fetus. A model of neurological cretinism. J. Clin. Invest. 1997; 99: 2701-2709.
40. Berbel P, Auso E, Garcia-Velasco JV, Molina ML, Camacho M. Role of thyroid hormones in the maturation and organisation of rat barrel cortex. Neuroscience 2001; 107: 383-394.
41. Berbel P, Guadano-Ferraz A, Martinez M, Quiles JA, Balboa R, Innocenti GM. Organization of auditory callosal connections in hypothyroid adult rats. Eur. J. Neurosci. 1993; 5: 1465-1478.
42. Berbel P, Guadaño-Ferraz A, Angulo A, Cerezo JR. Role of thyroid hormones in the maturation of interhemispheric connections in rat. Behav. Brain Res. 1994; 64: 9-14.
43. Marin-Padilla M. Three-dimensional structural organization of layer I of the human cerebral cortex: a Golgi study. J. Comp. Neurol. 1990; 299: 89-105.
44. Alvarez-Dolado M, Gonzalez-Moreno M, Valencia A, Zenke M, Bernal J, Muñoz A. Identification of a mammalian homologue of the fungal Tom70 mitochondrial precursor protein import receptor as a thyroid hormone-regulated gene in specific brain regions. J. Neurochem. 1999; 73: 2240-2249.
45. García-Fernández LF, Rausell E, Urade Y, Hayaishi O, Bernal J, Muñoz A. Hypothyroidism alters the expression of prostaglandin D2 synthase/beta trace in specific areas of the developing rat brain. Eur. J. Neurosci. 1997; 9: 1566-1573.
46. Del Rio JA, Heimrich B, Borrell V, Forster E, Drakew A, Alcantara S, Nakajima K, Miyata T, Ogawa M, Mikoshiba K, Derer P, Frotscher M, Soriano E. A role for Cajal-Retzius cells and reelin in the development of hippocampal connections. Nature 1997; 385: 70-74.
47. Ausó E, Lavado-Autric R, Cuevas E, Del Rey FE, Morreale De Escobar G, Berbel P. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 2004; 145: 4037-4047.
48. Goodman JH, Gilbert ME. Modest thyroid hormone insufficiency during development induces a cellular malformation in the corpus callosum: a model of cortical dysplasia. Endocrinology 2007; 148: 2593-2597.
49. Nicholson JL, Altman J. The effects of early hypo- and hyperthyroidism on the development of the rat cerebellar cortex. I. Cell proliferation and differentiation. Brain Res. 1972; 44: 13-23.
50. Balazs R, Brooksbank BWL, Davison AN, Eayrs JT, Wilson DA. The effect of neonatal thyroidectomy on myelination in the rat brain. Brain Res. 1969; 15: 219-232.
51. Malone MJ, Rosman NP, Szoke M, Davis D. Myelination of brain in experimental hypothyroidism. An electron-microscopic and biochemical study of purified myelin isolates. J. Neurol. Sci. 1975; 26: 1-11.
52. Noguchi T, Sugisaki T. Hypomyelination in the cerebrum of the congenitally hypothyroid mouse (hyt). J. Neurochem. 1984; 42: 891-893.
53. Adamo AM, Aloise PA, Soto EF, Pasquini JM. Neonatal Hyperthyroidism in the Rat Produces an Increase in the Activity of Microperoxisomal Marker Enzymes Coincident with Biochemical Signs of Accelerated Myelination. J. Neurosci. Res. 1990; 25: 353-359.
54. Notterpek LM, Rome LH. Functional evidence for the role of axolemma in CNS myelination. Neuron 1994; 13: 473-485.
55. Escobar-Morreale H, Obregón MJ, Escobar del Rey F, Morreale de Escobar G. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. J. Clin. Invest. 1995; 96: 2828-2838.
56. Escobar-Morreale H, Obregón MJ, Escobar del Rey F, Morreale de Escobar G. Tissue-specific patterns of changes in 3,5,3′-triiodo-L-thyronine concentrations in thyroidectomized rats infused with

increasing doses of the hormone. Which are the regulatory mechanisms? Biochimie 1999; 81: 453-462.
57. Calvo R, Obregón MJ, Ruiz de Oña C, Escobar del Rey F, Morreale de Escobar G. Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3′-triiodothyronine in the protection of the fetal brain. J. Clin. Invest. 1990; 86: 889-899.
58. Grijota-Martinez C, Diez D, Morreale de Escobar G, Bernal J, Morte B. Lack of action of exogenously administered T3 on the fetal rat brain despite expression of the monocarboxylate transporter 8. Endocrinology 2011; 152: 1713-1721.
59. Galton VA, Wood ET, St Germain EA, Withrow CA, Aldrich G, St Germain GM, Clark AS, St Germain DL. Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development. Endocrinology 2007; 148: 3080-3088.
60. Morreale de Escobar G, Obregón MJ, Escobar del Rey F. Fetal and maternal thyroid hormones. Horm. Res. 1987; 26: 12-27.
61. Morreale de Escobar G, Pastor R, Obregón MJ, Escobar del Rey F. Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues. Endocrinology 1985; 117: 1890-1901.
62. Nataf B, Sfez M. Debut du fonctionnement de la thyroide foetale du rat. Soc. Biol. 1961; 156: 1235-1238.
63. Obregón MJ, Mallol J, Pastor R, Morreale de Escobar G, Escobar del Rey G. L-thyroxine and 3,3′,5 -triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. Endocrinology 1984; 114: 305-307.
64. Porterfield SP, Hendrich CE. Tissue iodothyronine levels in fetuses of control and hypothyroid rats at 13 and 16 days gestation. Endocrinology 1992; 131: 195-200.
65. Woods RJ, Sinha AK, Ekins RP. Uptake and metabolism of thyroid hormones by the rat foetus early in pregnancy. Clin. Sci. 1984; 67: 359-363.
66. Morreale de Escobar G, Calvo R, Obregón MJ, Escobar del Rey F. Contribution of maternal thyroxine to fetal thyroxine pools in normal rats near term. Endocrinology 1990; 126: 2765-2767.
67. Contempré B, Jauniaux E, Calvo R, Jurkovic D, Campbell S, Morreale de Escobar G. Detection of thyroid hormones in human embryonic cavities during the first trimester of pregnancy. J. Clin. Endocrinol. Metabol. 1993; 77: 1719-1722.
68. Bernal J, Pekonen F. Ontogenesis of the nuclear 3,5,3′-triiodothyronine receptor in the human fetal brain. Endocrinology 1984; 114: 677-679.
69. Vulsma T, Gons MH, de Vijlder J. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid dysgenesis. N. Eng. J. Med. 1989; 321: 13-16.
70. Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeold A, Bianco AC. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocr Rev 2008; 29: 898-938.
71. Bianco AC, Larsen PR. Cellular and structural biology of the deiodinases. Thyroid 2005; 15: 777-786.
72. Friesema EC, Jansen J, Jachtenberg JW, Visser WE, Kester MH, Visser TJ. Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10. Mol Endocrinol 2008; 22: 1357-1369.
73. Dentice M, Bandyopadhyay A, Gereben B, Callebaut I, Christoffolete MA, Kim BW, Nissim S, Mornon JP, Zavacki AM, Zeold A, Capelo LP, Curcio-Morelli C, Ribeiro R, Harney JW, Tabin CJ, Bianco AC. The Hedgehog-inducible ubiquitin ligase subunit WSB-1 modulates thyroid hormone activation and PTHrP secretion in the developing growth plate. Nat Cell Biol 2005; 7: 698-705.

74. Dentice M, Luongo C, Huang S, Ambrosio R, Elefante A, Mirebeau-Prunier D, Zavacki AM, Fenzi G, Grachtchouk M, Hutchin M, Dlugosz AA, Bianco AC, Missero C, Larsen PR, Salvatore D. Sonic hedgehog-induced type 3 deiodinase blocks thyroid hormone action enhancing proliferation of normal and malignant keratinocytes. Proc Natl Acad Sci U S A 2007; 104: 14466-14471.
75. Larsen PR, Silva JR, Kaplan MM. Relationships between circulating and intracellular thyroid hormones. Physiological and clinical implications. Endocr. Rev. 1981; 2: 87-102.
76. van Doorn J, Roelfsema F, van der Heide D. Contribution from local conversion of thyroxine to 3,5,3′-triiodothyronine to intracellular 3,5,3′-triiodothyronine in several organs in hypothyroid rats at isotope equilibrium. Acta Endocrinol. (Copenh.) 1982; 101: 386-396.
77. Crantz FR, Silva JE, Larsen PR. An analysis of the sources and quantity of 3,5,3′-triiodothyronine specifically bound to nuclear receptors in rat cerebral cortex and cerebellum. Endocrinology 1982; 110: 367-375.
78. Bates JM, St.Germain DL, Galton VA. Expression profiles of the three iodothyronine deiodinases D1, D2 and D3, in the developing rat. Endocrinology 1999; 140: 844-851.
79. Ruiz de Oña C, Obregón MJ, Escobar del Rey F, Morreale de Escobar G. Developmental changes in rat brain 5′-deiodinase and thyroid hormones during the fetal period: the effects of fetal hypothyroidism and maternal thyroid hormones. Pediatr. Res. 1988; 24: 588-594.
80. Leonard JL, Kaplan MM, Visser TJ, Silva JE, Larsen PR. Cerebral cortex responds rapidly to thyroid hormones. Science 1981; 214: 571-573.
81. Obregón MJ, Ruiz de Oña C, Calvo R, Escobar del Rey F, Morreale de Escobar G. Outer ring iodothyronine deiodinases and thyroid hormone economy: responses to iodine deficiency in the rat fetus and neonate. Endocrinology 1991; 129: 2663-2673.
82. Farwell AP, Leonard JL. Dissociation of actin polymerization and enzyme inactivation in the hormonal regulation of type II iodothyronine 5′-deiodinase activity in astrocytes. Endocrinology 1992; 131: 721-728.
83. Farwell AP, DiBenedetto DJ, Leonard JL. Thyroxine targets different pathways of internalization of type II iodothyronine 5′-deiodinase in astrocytes. J. Biol. Chem. 1993; 268: 5055-5062.
84. Stachelek SJ, Kowalik TF, Farwell AP, Leonard JL. Myosin V plays an essential role in the thyroid hormone-dependent endocytosis of type II iodothyronine 5′-deiodinase. J Biol Chem 2000; 275: 31701-31707.
85. Steinsapir J, Bianco AC, Buettner C, Harney J, Larsen PR. Substrate-induced down-regulation of human type 2 deiodinase (hD2) is mediated through proteasomal degradation and requires interaction with the enzyme's active center. Endocrinology 2000; 141: 1127-1135.
86. Steinsapir J, Harney J, Larsen PR. Type 2 iodothyronine deiodinase in rat pituitary tumor cells is inactivated in proteasomes. J. Clin. Invest. 1998; 102: 1895-1899.
87. Burmeister LA, Pachucki J, St Germain DL. Thyroid hormones inhibit type 2 iodothyronine deiodinase in the rat cerebral cortex by both pre- and posttranslational mechanisms. Endocrinology 1997; 138: 5231-5237.
88. Croteau W, Davey JC, Galton VA, St. Germain DL. Cloning of the mammalian type II iodothyronine deiodinase: a selenoprotein differentially expressed and regulated in the human brain and other tissues. J. Clin. Invest. 1996; 98: 405-417.
89. Guadaño-Ferraz A, Escámez MJ, Rausell E, Bernal J. Expression of type 2 iodothyronine deiodinase in hypothyroid rat brain indicates an important role of thyroid hormone in the development of specific primary sensory systems. J. Neurosci. 1999; 19: 3430-3439.
90. Tu HM, Kim SW, Salvatore D, Bartha T, Legradi G, Larsen PR, Lechan RM. Regional distribution of

type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. Endocrinology 1997; 138: 3359-3368.
91. Riskind PN, Kolodny JM, Larsen PR. The regional distribution of type II 5′-monodeiodinase in euthyroid and hypothyroid rats. Brain Res. 1987; 420: 194-198.
92. Guadaño-Ferraz A, Obregón MJ, St-Germain D, Bernal J. The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. Proc. Natl. Acad. Sci. USA 1997; 94: 10391-10396.
93. Rodriguez EM, Gonzalez CB, Delannoy L. Cellular organization of the lateral and postinfundibular regions of the median eminence in the rat. Cell Tissue Res. 1979; 201: 377-408.
94. Lechan RM, Fekete C. Infundibular tanycytes as modulators of neuroendocrine function: hypothetical role in the regulation of the thyroid and gonadal axis. Acta Biomed 2007; 78 Suppl 1: 84-98.
95. Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, Yasuo S, Katou Y, Kageyama S, Uno Y, Kasukawa T, Iigo M, Sharp PJ, Iwasawa A, Suzuki Y, Sugano S, Niimi T, Mizutani M, Namikawa T, Ebihara S, Ueda HR, Yoshimura T. Thyrotrophin in the pars tuberalis triggers photoperiodic response. Nature 2008; 452: 317-322.
96. Ono H, Hoshino Y, Yasuo S, Watanabe M, Nakane Y, Murai A, Ebihara S, Korf HW, Yoshimura T. Involvement of thyrotropin in photoperiodic signal transduction in mice. Proc Natl Acad Sci U S A 2008; 105: 18238-18242.
97. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, Thompson WJ, Barres BA. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci 2008; 28: 264-278.
98. Fliers E, Unmehopa UA, Alkemade A. Functional neuroanatomy of thyroid hormone feedback in the human hypothalamus and pituitary gland. Mol Cell Endocrinol 2006; 251: 1-8.
99. Bradley DJ, Towle HC, Young WS. Spatial and temporal expression of a- and b- thyroid hormone receptor mRNAs, including the b2-subtype, in the developing mammalian nervous system. J. Neurosci. 1992; 12: 2288-2302.
100. Mellström B, Naranjo JR, Santos A, González AM, Bernal J. Independent expression of the a and b c-erbA genes in developing rat brain. Mol. Endocrinol. 1991; 5: 1339-1350.
101. Tsacopoulos M, Magistretti PJ. Metabolic coupling between glia and neurons. J. Neurosci. 1996; 16: 877-885.
102. Campos-Barros A, Amma LL, Faris JS, Shailam R, Kelley MW, Forrest D. Type 2 iodothyronine deiodinase expression in the cochlea before the onset of hearing. Proc. Natl. Acad. Sci. USA 2000; 97: 1287-1292.
103. Bradley DJ, Towle HC, Young WSI. Alpha and beta thyroid hormone receptor (TR) gene expression during auditory neurogenesis: evidence for TR isoform-specific transcriptional regulation in vivo. Proc. Natl. Acad. Sci. USA 1994; 91: 439-443.
104. Lauterman J, ten Cate W-JF. Postnatal expression of the rat alpha-thyroid hormone receptor in the rat cochlea. Hearing Res. 1997; 107: 23-28.
105. Morte B, Ceballos A, Diez D, Grijota-Martinez C, Dumitrescu AM, Di Cosmo C, Galton VA, Refetoff S, Bernal J. Thyroid hormone-regulated mouse cerebral cortex genes are differentially dependent on the source of the hormone: a study in monocarboxylate transporter-8- and deiodinase-2-deficient mice. Endocrinology 2010; 151: 2381-2387.
106. Hernandez A, Morte B, Belinchon MM, Ceballos A, Bernal J. Critical role of types 2 and 3

deiodinases in the negative regulation of gene expression by T(3)in the mouse cerebral cortex. Endocrinology 2012; 153: 2919-2928.
107. Galton VA, Schneider MJ, Clark AS, St Germain DL. Life without T4 to T3 conversion: Studies in mice devoid of the 5′-deiodinases. Endocrinology 2009.
108. St Germain DL. Thyroid hormone deiodination. Curr. Op. Endocrinol. Diabetes 1995; 2: 421-428.
109. Kaplan MM, Yaskoski KA. Maturational patterns of iodothyronine phenolic and tyrosyl ring deiodinase activities in rat cerebrum, cerebellum, and hypothalamus. J. Clin. Invest. 1981; 67: 1208-1214.
110. Santini F, Chiovato L, Ghirri P, Lapi P, Mammoli C, Montanelli L, Scartabelli G, Ceccarini G, Coccoli L, Chopra IJ, Boldrini A, Pinchera A. Serum iodothyronines in the human fetus and the newborn: evidence for an important role of placenta in fetal thyroid hormone homeostasis. J. Clin. Endocrinol. Metabol. 1999; 84: 493-498.
111. Schröder-van der Elst JP, Van der Heide D, Morreale de Escobar G, Obregón MJ. Iodothyronine deiodinase activities in fetal rat tissues at several levels of iodine deficiency: a role for the skin in 3,5,3′-triiodothyronine economy? Endocrinology 1998; 139: 2229-2234.
112. Kaplan MM, Shaw EA. Type II iodothyronine 5′-deiodination by human and rat placenta in vitro. J. Clin. Endocrinol. Metabol. 1984; 59: 253-257.
113. Koopdonk-Kool JM, De Vijlder JJ, Veenboer GJM, Ris-Stalpers C, Kok JH, Vulsma T, Boer K, Visser TJ. Type II and type III deiodinase activity in human placenta as a function of gestational age. J. Clin. Endocrinol. Metabol. 1996; 81: 2154-2158.
114. Courtin F, Liva P, Gavaret JM, Toru Delbauffe D, Pierre M. Induction of 5-deiodinase activity in astroglial cells by 12-O-tetradecanoylphorbol 13-acetate and fibroblast growth factors. J. Neurochem. 1991; 56: 1107-1113.
115. Esfandiari A, Courtin F, Lennon AM, Gavaret JM, Pierre M. Induction of type III deiodinase activity in astroglial cells by thyroid hormones. Endocrinology 1992; 131: 1682-1688.
116. Esfandiari A, Gagelin C, Gavaret JM, Pavelka S, Lennon AM, Pierre M, Courtin F. Induction of type III-deiodinase activity in astroglial cells by retinoids. Glia 1994; 11: 255-261.
117. Escámez MJ, Guadaño-Ferraz A, Cuadrado A, Bernal J. Type 3 iodothyronine deiodinase is selectively expressed in areas related to sexual differentiation in the newborn rat brain. Endocrinology 1999; 140: 5443-5446.
118. Tu HM, Legradi G, Bartha T, Salvatore D, Lechan RM, Larsen PR. Regional expression of the type 3 iodothyronine deiodinase messenger ribonucleic acid in the rat central nervous system and its regulation by thyroid hormone. Endocrinology 1999; 140: 784-790.
119. Becker KB, Stephens KC, Davey JC, Schneider MJ, Galton VA. The type 2 and type 3 iodothyronine deiodinases play important roles in coordinating development in Rana catesbeiana tadpoles. Endocrinology 1997; 138: 2989-2997.
120. Berry DL, Rose CS, Remo BF, Brown DD. The expression pattern of thyroid hormone response genes in remodeling tadpole tissues defines distinct growth and resorption gene expression programs. Dev. Biol. 1998; 203: 24-35.
121. Huang H, Marsh-Armstrong N, Brown DD. Metamorphosis is inhibited in transgenic xenopus laevis tadpoles that overexpress type III deiodinase. Proc. Natl. Acad. Sci. USA 1998; 96: 962-967.
122. Marsh-Armstrong N, Huang H, Remo BF, Liu TT, Brown DD. Asymmetric growth and development of the xenopus laevis retina during metamorphosis is controlled by type III deiodinase. Neuron 1999; 24: 871-878.
123. Mortimer RH, Galligan JP, Cannell GR, Addison RS, Roberts MS. Maternal to fetal thyroxine

transmission in the human term placenta is limited by inner ring deiodination. J. Clin. Endocrinol. Metabol. 1996; 81: 2247-2249.
124. Galton VA, Martínez E, Hernández A, St. Germain EA, Bates JM, St.Germain DL. Pregnant rat uterus expresses high levels of the type 3 iodothyronine deiodinase. J. Clin. Invest. 1999; 103: 979-987.
125. Baum MJ. Psychosexual development. In Zigmond MJ, Bloom FE, Landis SC et al. (eds): Fundamental Neuroscience, Edition San Diego: Academic Press 1999; 1229-1244.
126. Dellovade TL, Zhu YS, Krey L, Pfaff DW. Thyroid hormone and estrogen interact to regulate behavior. Proc. Natl. Acad. Sci. USA 1996; 93: 12581-12586.
127. Hernandez A, Martinez ME, Fiering S, Galton VA, St Germain D. Type 3 deiodinase is critical for the maturation and function of the thyroid axis. J Clin Invest 2006; 116: 476-484.
128. Hernandez A, Martinez ME, Liao XH, Van Sande J, Refetoff S, Galton VA, St Germain DL. Type 3 deiodinase deficiency results in functional abnormalities at multiple levels of the thyroid axis. Endocrinology 2007; 148: 5680-5687.
129. Hernandez A, Quignodon L, Martinez ME, Flamant F, St Germain DL. Type 3 deiodinase deficiency causes spatial and temporal alterations in brain T3 signaling that are dissociated from serum thyroid hormone levels. Endocrinology 2010; 151: 5550-5558.
130. Barca-Mayo O, Liao XH, Alonso M, Di Cosmo C, Hernandez A, Refetoff S, Weiss RE. Thyroid hormone receptor alpha and regulation of type 3 deiodinase. Mol Endocrinol 2011; 25: 575-583.
131. Freitas BC, Gereben B, Castillo M, Kallo I, Zeold A, Egri P, Liposits Z, Zavacki AM, Maciel RM, Jo S, Singru P, Sanchez E, Lechan RM, Bianco AC. Paracrine signaling by glial cell-derived triiodothyronine activates neuronal gene expression in the rodent brain and human cells. J Clin Invest 2010; 120: 2206-2217.
132. Ceballos A, Belinchon MM, Sanchez-Mendoza E, Grijota-Martinez C, Dumitrescu AM, Refetoff S, Morte B, Bernal J. Importance of Monocarboxylate transporter 8 (Mct8) for the blood-brain barrier dependent availability of 3,5,3'-Triiodo-L-Thyronine (T3). Endocrinology 2009; In Press.
133. Laterra J, Goldstein GW. Ventricular organization of cerebrospinal fluid: blood-brain barrier, brain edema and hydrocephalus. In Kandel ER, Schwartz JH, Jessell TM (eds): Principles of Neural Science, Edition New York: McGraw-Hill 2000; 1288-1301.
134. Kacem K, Lacombe P, Seylaz J, Bonvento G. Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: a confocal microscopy study. Glia 1998; 23: 1-10.
135. Mathiisen TM, Lehre KP, Danbolt NC, Ottersen OP. The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. Glia 2010; 58: 1094-1103.
136. Hagen GA, Solberg LA. Brain and cerebrospinal fluid permeability to intravenous thyroid hormones. Endocrinology 1974; 95: 1398-1410.
137. Hagen G, Elliot WJ. Transport of thyroid hormones in serum and cerebrospinal fluid. J. Clin. Endocrinol. Metabol. 1973; 37: 415-422.
138. Kirkegaard C, Faber J. Free thyroxine and 3,3′,5′-triiodothyronine levels in cerebrospinal fluid in patients with endogenous depression. Acta Endocrinol. (Copenh.) 1991; 124: 166-172.
139. Thompson PJ, Burman KD, Wright FD, Potter MW, Wartofsky L. Iodothyronine levels in human cerebrospinal fluid. J. Clin. Endocrinol. Metabol. 1982; 54: 653-655.
140. Dickson PW, Aldred AR, Menting JGT, Marley PD, Sawyer WH, Schreiber G. Thyroxine transport in choroid plexus. J. Biol. Chem. 1987; 262: 13907-13915.

141. Schreiber G, Southwell BR, Richardson SJ. Hormone delivery systems to the brain-transthyretin. Exper. Clin. Endocrinol. Diab. 1995; 103: 75-80.
142. Palha JA, Episkopou V, Maeda S, Shimada K, Gottesman ME. Thyroid hormone metabolism in a transthyretin-null mouse strain. J. Biol. Chem. 1994; 269: 33135-33139.
143. Palha JA, Hays MT, Morreale de Escobar G, Episkopou V, Gottesman ME, Saraiva MJ. Transthyretin is not essential for thyroxine to reach the brain and other tissues in transthyretin-null mice. Am. J. Physiol. 1997; 272: E485-493.
144. Chanoine J-P, Alex S, Fang SL, Stone S, Leonard JL, Köhrle J, Braverman LE. Role of transthyretin in the transport of thyroxine from the blood to the choroid plexus, the cerebrospinal fluid, and the brain. Endocrinology 1992; 130: 933-938.
145. Dratman MB, Crutchfield FL, Schoenhoff MB. Transport of iodothyronines from bloodstream to brain: contributions by blood:brain and choroid plexus:cerebrospinal fluid barriers. Brain Res. 1991; 554: 229-236.
146. Kendall JW, Jacobs JJ, Kramer RM. Studies on the transport of hormones from the cerebrospinal fluid to hypothalamus and pituitary. In Brain-Endocrine Interaction. Median Eminence: Structure and Function, Edition Munich: Karger, Basel 1972 1971; 342-349.
147. Bernal J. Thyroid hormone transport in developing brain. Curr Opin Endocrinol Diabetes Obes 2011; 18: 295-299.
148. Braun D, Wirth EK, Schweizer U. Thyroid hormone transporters in the brain. Rev Neurosci 2010; 21: 173-186.
149. Friesema EC, Visser WE, Visser TJ. Genetics and phenomics of thyroid hormone transport by MCT8. Mol Cell Endocrinol 2010; 322: 107-113.
150. Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. Mol Endocrinol 2011; 25: 1-14.
151. Halestrap AP. The monocarboxylate transporter family–Structure and functional characterization. IUBMB Life 2011; 64: 1-9.
152. Halestrap AP, Wilson MC. The monocarboxylate transporter family–role and regulation. IUBMB Life 2012; 64: 109-119.
153. Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. Am J Hum Genet 2004; 74: 168-175.
154. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH, Kuiper GG, Balkassmi S, Uitterlinden AG, Koehrle J, Rodien P, Halestrap AP, Visser TJ. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. Lancet 2004; 364: 1435-1437.
155. Friesema EC, Jansen J, Milici C, Visser TJ. Thyroid hormone transporters. Vitam Horm 2005; 70: 137-167.
156. Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, Grindstaff KK, Mengesha W, Raman C, Zerangue N. Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. Endocrinology 2008; 149: 6251-6261.
157. Heuer H, Maier MK, Iden S, Mittag J, Friesema EC, Visser TJ, Bauer K. The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. Endocrinology 2005; 146: 1701-1706.
158. Pérez-Castillo A, Bernal J, Ferreiro B, Pans T. The early ontogenesis of thyroid hormone receptor

in the rat fetus. Endocrinology 1985; 117: 2457-2461.
159. Schwartz HL, Oppenheimer JH. Ontogenesis of 3,5,3′-triiodothyronine receptors in neonatal rat brain, dissociation between receptor concentration and stimulation of oxygen consumption by 3,5,3′-triiodothyronine. Endocrinology 1978; 103: 943-948.
160. Strait KA, Schwartz HL, Perezcastillo A, Oppenheimer JH. Relationship of C-erbA Messenger RNA Content to Tissue Triiodothyronine Nuclear Binding Capacity and Function in Developing and Adult Rats. J. Biol.Chem. 1990; 265: 10514-10521.
161. Ferreiro B, Pastor R, Bernal J. T3 receptor occupancy and T3 levels in plasma and cytosol during rat brain development. Acta Endocrinol. (Copenh.) 1990; 123: 95-99.
162. Bradley DJ, Young III WS, Weinberger C. Differential expression of alpha and beta thyroid hormone receptor genes in rat brain and pituitary. Proc. Natl. Acad. Sci. USA 1989; 86: 72507254.
163. Wallis K, Dudazy S, van Hogerlinden M, Nordstrom K, Mittag J, Vennstrom B. The thyroid hormone receptor alpha1 protein is expressed in embryonic postmitotic neurons and persists in most adult neurons. Mol Endocrinol 2010; 24: 1904-1916.
164. Hodin RA, Lazar MA, Wintman BI, Darling DS, Koening RJ, Larsen PR, Moore DD, Chin WW. Identification of a thyroid hormone receptor that is pituitary-specific. Science (Wash.) 1989; 244: 76-79.
165. Schwartz HL, Lazar MA, Oppenheimer JH. Widespread distribution of immunoreactive thyroid hormone b2 receptor (TRb2) in the nuclei of extrapituitary rat tissues. J. Biol. Chem. 1994; 269: 24777-24782.
166. Lechan RM, Qi Y, Berrodin TJ, Davis KD, Schwartz HL, Strait KA, Oppenheimer JH, Lazar MA. Immunocytochemical delineation of thyroid hormone receptor b2-like immunoreactivity in the rat central nervous system. Endocrinology 1993; 132: 2461-2469.
167. Ercan-Fang S, Schwartz HL, Oppenheimer JH. Isoform specific 3,5,3′-triiodothyronine receptor binding capacity and messenger ribonucleic acid content in rat adenohypophysis: Effect of thyroidal state and comparison with extrapituitary tissues. Endocrinology 1996; 137: 3228-3233.
168. Iskaros J, Pickard M, Evans I, Sinha A, Hardiman P, Ekins R. Thyroid hormone receptor gene expression in first trimester human fetal brain. J. Clin. Endocrinol. Metabol. 2000; 85: 2620-2623.
169. Dobbing J, Sands J. Timing of neuroblast multiplication in developing human brain. Nature 1970; 226: 639-640.
170. Ferreiro B, Bernal J, Goodyer CG, Branchard CL. Estimation of nuclear thyroid hormone receptor saturation in human fetal brain and lung during early gestation. J. Clin. Endocrinol. Metabol. 1988; 67: 853-856.
171. Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TJ, Hume R, Morreale de Escobar G. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. J Clin Endocrinol Metab 2004; 89: 3117-3128.
172. Luo M, Faure R, Dussault JH. Ontogenesis of nuclear T3 receptors in primary cultured astrocytes and neurons. Brain Res. 1986; 381: 275-280.
173. Yusta B, Besnard F, Ortiz-Caro J, Pascual A, Aranda A, Sarlieve L. Evidence for the presence of nuclear 3,5,3′-triiodothyronine receptors in secondary cultures of pure rat oligodendrocytes. Endocrinology 1988; 122: 2278-2284.
174. Glauser L, Barakat-Walter I. Differential distribution of thyroid hormone receptor isoform in rat dorsal root ganglia and sciatic nerve in vivo and in vitro. J. Neuroendocrinol. 1997; 9: 217-227.
175. Barakat-Walter I, Droz B. Nuclear and cytoplasmic triiodothyronine binding sites in primary sensory neurons and Schwann cells: radioautographic study during development. J. Neuroendocrinol. 1995; 7: 127-136.

176. Carlson DJ, Strait KA, Schwartz HL, Oppenheimer JH. Immunofluorescent localization of thyroid hormone receptor isoforms in glial cells of rat brain. Endocrinology 1994; 135: 1831-1836.
177. Kolodny JM, Leonard JL, Larsen PR, Silva JE. Studies of nuclear 3,5,3′-triiodothyronine binding in primary cultures of rat brain. Endocrinology 1985; 117: 1848-1857.
178. Leonard JL, Farwell AP, Yen PM, Chin WW, Stula M. Differential expression of thyroid hormone receptor isoforms in neurons and astroglial cells. Endocrinology 1994; 135: 548-555.
179. Leonard JL. Non-genomic actions of thyroid hormone in brain development. Steroids 2008; 73: 1008-1012.
180. Iñiguez MA, De Lecea L, Guadaño-Ferraz A, Morte B, Gerendasy D, Sutcliffe JG, Bernal J. Cell-specific effects of thyroid hormone on RC3/neurogranin expression in rat brain. Endocrinology 1996; 137: 1032-1041.
181. Diez D, Grijota-Martinez C, Agretti P, De Marco G, Tonacchera M, Pinchera A, de Escobar GM, Bernal J, Morte B. Thyroid hormone action in the adult brain: gene expression profiling of the effects of single and multiple doses of triiodo-L-thyronine in the rat striatum. Endocrinology 2008; 149: 3989-4000.
182. Dong H, Wade M, Williams A, Lee A, Douglas GR, Yauk C. Molecular insight into the effects of hypothyroidism on the developing cerebellum. Biochem Biophys Res Commun 2005; 330: 1182-1193.
183. Martel J, Cayrou C, Puymirat J. Identification of new thyroid hormone-regulated genes in rat brain neuronal cultures. Neuroreport 2002; 13: 1849-1851.
184. Miller LD, McPhie P, Suzuki H, Kato Y, Liu ET, Cheng SY. Multi-tissue gene-expression analysis in a mouse model of thyroid hormone resistance. Genome Biol 2004; 5: R31.
185. Poguet AL, Legrand C, Feng X, Yen PM, Meltzer P, Samarut J, Flamant F. Microarray analysis of knockout mice identifies cyclin D2 as a possible mediator for the action of thyroid hormone during the postnatal development of the cerebellum. Dev Biol 2003; 254: 188-199.
186. Quignodon L, Grijota-Martinez C, Compe E, Guyot R, Allioli N, Laperriere D, Walker R, Meltzer P, Mader S, Samarut J, Flamant F. A combined approach identifies a limited number of new thyroid hormone target genes in post-natal mouse cerebellum. J Mol Endocrinol 2007; 39: 17-28.
187. Chatonnet F, Guyot R, Picou F, Bondesson M, Flamant F. Genome-wide search reveals the existence of a limited number of thyroid hormone receptor alpha target genes in cerebellar neurons. PLoS One 2012; 7: e30703.
188. Schwartz HL, Ross ME, Oppenheimer JH. Lack of effect of thyroid hormone on late fetal rat brain development. Endocrinology 1997; 138: 3119-3124.
189. Krebs J, Means RL, Honegger P. Induction of calmodulin kinase IV by the thyroid hormone during the development of rat brain. J. Biol. Chem. 1996; 271: 11055-11058.
190. Sampson D, Pickard MR, Sinha AK, Evans IM, Leonard AJ, Ekins RP. Maternal thyroid status regulates the expression of neuronal and astrocytic cytoskeletal proteins in the fetal brain. J Endocrinol 2000; 167: 439-445.
191. Dowling AL, Iannacone EA, Zoeller RT. Maternal Hypothyroidism Selectively Affects the Expression of Neuroendocrine-Specific Protein A Messenger Ribonucleic Acid in the Proliferative Zone of the Fetal Rat Brain Cortex. Endocrinology 2001; 142: 390-399.
192. Sinha RA, Pathak A, Mohan V, Bandyopadhyay S, Rastogi L, Godbole MM. Maternal thyroid hormone: a strong repressor of neuronal nitric oxide synthase in rat embryonic neocortex. Endocrinology 2008; 149: 4396-4401.
193. Morte B, Diez D, Auso E, Belinchon MM, Gil-Ibanez P, Grijota-Martinez C, Navarro D, de Escobar GM, Berbel P, Bernal J. Thyroid hormone regulation of gene expression in the developing rat fetal

cerebral cortex: prominent role of the Ca2+/calmodulin-dependent protein kinase IV pathway. Endocrinology 2010; 151: 810-820.
194. Krebs J, Honegger P. Calmodulin kinase IV: expression and function during rat brain development. Biochim. Biophys. Acta 1996; 1313: 217-222.
195. Liu YY, Brent GA. Thyroid hormone-dependent gene expression in differentiated embryonic stem cells and embryonal carcinoma cells: identification of novel thyroid hormone target genes by deoxyribonucleic acid microarray analysis. Endocrinology 2005; 146: 776-783.
196. Sutcliffe JG. The genes for myelin revisited. Trends Genet. 1988; 4: 211-213.
197. Ibarrola N, Rodriguez-Peña A. Hypothyroidism coordinately and transiently affects myelin proein gene expression in most rat brain regions during postnatal development. Brain Res. 1997; 752: 285-293.
198. Rodriguez-Pena A, Ibarrola N, Iniguez MA, Munoz A, Bernal J. Neonatal hypothyroidism affects the timely expression of myelin- associated glycoprotein in the rat brain. J Clin Invest 1993; 91: 812-818.
199. Schoonover CM, Seibel MM, Jolson DM, Stack MJ, Rahman RJ, Jones SA, Mariash CN, Anderson GW. Thyroid hormone regulates oligodendrocyte accumulation in developing rat brain white matter tracts. Endocrinology 2004; 145: 5013-5020.
200. Gao FB, Apperly J, Raff M. Cell-intrinsic timers and thyroid hormone regulate the probability of cell -cycle withdrawal and differentiation of oligodendrocyte precursor cells. Dev Biol 1998; 197: 54-66.
201. Nygard M, Wahlstrom GM, Gustafsson MV, Tokumoto YM, Bondesson M. Hormone-Dependent Repression of the E2F-1 Gene by Thyroid Hormone Receptors. Mol Endocrinol 2003; 17: 79-92.
202. Billon N, Jolicoeur C, Tokumoto Y, Vennstrom B, Raff M. Normal timing of oligodendrocyte development depends on thyroid hormone receptor alpha 1 (TRalpha1). Embo J 2002; 21: 6452-6460.
203. Billon N, Tokumoto Y, Forrest D, Raff M. Role of thyroid hormone receptors in timing oligodendrocyte differentiation. Dev Biol 2001; 235: 110-120.
204. Wrutniak-Cabello C, Casas F, Cabello G. Thyroid hormone action in mitochondria. J Mol Endocrinol 2001; 26: 67-77.
205. Barsano CP, DeGroot LJ, Getz GS. The effect of thyroid hormone on in vivo rat liver mitochondria RNA synthesis. Endocrinology 1977; 100: 52-60.
206. Enriquez JA, Fernandez-Silva P, Garrido-Perez N, Lopez-Perez MJ, Perez-Martos A, Montoya J. Direct regulation of mitochondrial RNA synthesis by thyroid hormone. Mol. Cell. Biol. 1999; 19: 657-670.
207. Vega-Núñez E, Alvarez AM, Menendez-Hurtado A, Santos A, Pérez-Castillo A. Neuronal mitochondrial morphology and transmembrane potential are severely altered by hypothyroidism during rat brain development. Endocrinology 1997; 138: 3771-3778.
208. Vega-Núñez E, Menéndez-Hurtado A, Garesse R, Santos A, Perez-Castillo A. Thyroid hormone-regulated brain mitochondrial genes revealed by differential cDNA cloning. J. Clin. Invest. 1995; 96: 893-899.
209. Koibuchi N, Matsuzaki S, Ichimura K, Ohtake H, Yamaoka S. Ontogenic changes in the expression of cytochrome c oxidase subunit I gene in the cerebellar cortex of the perinatal hypothyroid rat. Endocrinology 1996; 137: 5096-5108.
210. Iglesias T, Caubín J, Zaballos A, Bernal J, Muñoz A. Identification of the mitochondrial NADH dehydrogenase subunit 3 (ND3) as a thyroid hormone regulated gene by whole genome PCR analysis. Biochem. Biophys. Res. Comm. 1995; 210: 995-1000.
211. Alvarez-Dolado M, Ruiz M, del Rio JA, Alcantara S, Burgaya F, Sheldon M, Nakajima K, Bernal J,

Howell BW, Curran T, Soriano E, Muñoz A. Thyroid hormone regulates reelin and dab1 expression during brain development. J. Neurosci. 1999; 19: 6979-6973.
212. Alvarez-Dolado M, Cuadrado A, Navarro-Yubero C, Sonderegger P, Furley AJ, Bernal J, Muñoz A. Regulation of the L1 cell adhesion molecule by thyroid hormone in the developing brain. Mol. Cell. Neurobiol. 2000; 499-514.
213. Alvarez-Dolado M, Gonzalez-Sancho JM, Bernal J, Muñoz A. Developmental expression of tenascin-C is altered by hypothyroidism in the rat brain. Neurosci. 1998; 84: 309-322.
214. Farwell AP, Dubord-Tomasetti SA. Thyroid hormone regulates the expression of laminin in the developing rat cerebellum. Endocrinology 1999; 140: 4221-4227.
215. Pérez-Juste G, Aranda A. The cyclin-dependent kinase inhibitor p27(Kip1) is involved in thyroid hormone-mediated neuronal differentiation. J. Biol. Chem. 1999; 19: 5026-5031.
216. Qi JS, Yuan Y, Desai-Yajnik V, Samuels HH. Regulation of the mdm2 oncogene by thyroid hormone receptor. Mol Cell Biol 1999; 19: 864-872.
217. Wood WM, Sarapura VD, Dowding JM, Woodmansee WW, Haakinson DJ, Gordon DF, Ridgway EC. Early gene expression changes preceding thyroid hormone-induced involution of a thyrotrope tumor. Endocrinology 2002; 143: 347-359.
218. Aniello F, Couchie D, Gripois D, Nunez J. Regulation of five tubulin isotypes by thyroid hormone during brain development. J. Neurochem. 1991; 57: 1781-1786.
219. Lorenzo PI, Menard C, Miller FD, Bernal J. Thyroid hormone-dependent regulation of Ta1 a-tubulin during brain development. Mol Cell Neurosci 2002; 19: 333-343.
220. Silva JE, Rudas P. Effects of congenital hypothyroidism on microtubule-associated protein-2 expression in the cerebellum of the rat. Endocrinology 1990; 126: 1276-1282.
221. Aniello F, Couchie D, Bridoux AM, Gripois D, Nunez J. Splicing of juvenile and adult tau mRNA variants is regulated by thyroid hormone. Proc. Natl. Acad. Sci. U S A 1991; 88: 4035-4039.
222. Lima FR, Goncalves N, Gomes FC, de Freitas MS, Moura Neto V. Thyroid hormone action on astroglial cells from distinct brain regions during development. Int J Dev Neurosci 1998; 16: 19-27.
223. Manzano J, Bernal J, Morte B. Influence of thyroid hormones on maturation of rat cerebellar astrocytes. Int J Dev Neurosci 2007; 25: 171-179.
224. Clos J, Legrand C, Legrand J. Effects of thyroid state on the formation and early morphological development of bergmann glia in the developing rat cerebellum. Dev Neurosci 1980; 3: 199-208.
225. Lima FR, Gervais A, Colin C, Izembart M, Neto VM, Mallat M. Regulation of microglial development: a novel role for thyroid hormone. J Neurosci 2001; 21: 2028-2038.
226. Gharami K, Das S. Thyroid hormone-induced morphological differentiation and maturation of astrocytes are mediated through the beta-adrenergic receptor. J Neurochem 2000; 75: 1962-1969.
227. Alvarez-Dolado M, Iglesias T, Rodríguez-Peña A, Bernal J, Muñoz A. Expression of neurotrophins and the trk family of neurotrophin receptors in normal and hypothyroid rat brain. Mol. Brain Res. 1994; 27: 249-257.
228. Lindholm D, Castren E, Tsoulfas P, Kolbeck R, Penha Berzaghi M, Leingartner A, Heisenberg CP, Tesarollo L, parada LF, Thoenen H. Neurotrophin-3 induced by tri-iodothyronine in cerebellar granullar cells promotes purkinje cell differentiation. J. Cell Biol. 1993; 122: 443-450.
229. Gerendasy DD, Sutcliffe JG. RC3/neurogranin, a postsynaptic calpacitin for setting the response threshold to calcium influxes. Mol. Neurobiol. 1997; 15: 131-163.
230. Zhong L, Gerges NZ. Neurogranin targets calmodulin and lowers the threshold for the induction of long-term potentiation. PLoS One 2012; 7: e41275.
231. Miyakawa T, Yared E, Pak JH, Huang FL, Huang KP, Crawley JN. Neurogranin null mutant mice

display performance deficits on spatial learning tasks with anxiety related components. Hippocampus 2001; 11: 763-775.
232. Martinez de Arrieta C, Morte B, Coloma A, Bernal J. The human RC3 gene homolog, NRGN contains a thyroid hormone-responsive element located in the first intron. Endocrinology 1999; 140: 335 -343.
233. Vargiu P, De Abajo R, Garcia-Ranea JA, Valencia A, Santisteban P, Crespo P, Bernal J. The small GTP-binding protein, Rhes, regulates signal transduction from G protein-coupled receptors. Oncogene 2004; 23: 559-568.
234. Vargiu P, Morte B, Manzano J, Perez J, de Abajo R, Gregor Sutcliffe J, Bernal J. Thyroid hormone regulation of rhes, a novel Ras homolog gene expressed in the striatum. Brain Res Mol Brain Res 2001; 94: 1-8.
235. Bang S, Steenstra C, Kim SF. Striatum specific protein, Rhes regulates AKT pathway. Neurosci Lett 2012; 521: 142-147.
236. Subramaniam S, Napolitano F, Mealer RG, Kim S, Errico F, Barrow R, Shahani N, Tyagi R, Snyder SH, Usiello A. Rhes, a striatal-enriched small G protein, mediates mTOR signaling and L-DOPA -induced dyskinesia. Nat Neurosci 2011; 15: 191-193.
237. Subramaniam S, Sixt KM, Barrow R, Snyder SH. Rhes, a striatal specific protein, mediates mutant -huntingtin cytotoxicity. Science 2009; 324: 1327-1330.
238. Luo Y, Denker BM. Interaction of heterotrimeric G protein Galphao with Purkinje cell protein-2. Evidence for a novel nucleotide exchange factor. J Biol Chem 1999; 274: 10685-10688.
239. Koritschoner NP, Alvarez-Dolado M, Kurz SM, Heikenwalder MF, Hacker C, Vogel F, Munoz A, Zenke M. Thyroid hormone regulates the obesity gene tub. EMBO Rep 2001; 2: 499-504.
240. Santagata S, Boggon TJ, Baird CL, Gomez CA, Zhao J, Shan WS, Myszka DG, Shapiro L. G-protein signaling through tubby proteins. Science 2001; 292: 2041-2050.
241. Mellström B, Pipaon C, Naranjo JR, Perez-Castillo A, Santos A. Differential effect of thyroid hormone on NGFI-A gene expression in developing rat brain. Endocrinology 1994; 135: 583-588.
242. Ghorbel MT, Seugnet I, Hadj-Sahraoui N, Topilko P, Levi G, Demeneix B. Thyroid hormone effects on Krox-24 transcription in the post-natal mouse brain are developmentally regulated but are not correlated with mitosis. Oncogene 1999; 18: 917-924.
243. Cayrou C, Denver RJ, Puymirat J. Suppression of the basic transcription element-binding protein in brain neuronal cultures inhibits thyroid hormone-induced neurite branching. Endocrinology 2002; 143: 2242-2249.
244. Denver RJ, Ouellet L, Furling D, Kobayashi A, Fujii-Kuriyama Y, Puymirat J. Basic transcription element-binding protein (BTEB) is a thyroid hormone-regulated gene in the developing central nervous system. Evidence for a role in neurite outgrowth. J. Biol. Chem. 1999; 274: 23128-23134.
245. Avci HX, Lebrun C, Wehrle R, Doulazmi M, Chatonnet F, Morel MP, Ema M, Vodjdani G, Sotelo C, Flamant F, Dusart I. Thyroid hormone triggers the developmental loss of axonal regenerative capacity via thyroid hormone receptor alpha1 and kruppel-like factor 9 in Purkinje cells. Proc Natl Acad Sci U S A 2012; 109: 14206-14211.
246. Koibuchi N, Chin WW. RORa gene expression in the perinatal rat cerebellum: ontogeny and thyroid hormone regulation. Endocrinology 1998; 139: 2335-2341.
247. Thompson CC. Thyroid hormone-responsive genes in developing cerebellum include a novel synaptotagmin and a hairless homolog. J. Neurosci. 1996; 16: 7832-7840.
248. Thompson CC, Bottcher MC. The product of a thyroid hormone-responsive gene interacts with thyroid hormone receptors. Proc Natl Acad Sci U S A 1997; 94: 8527-8532.

249. Cuadrado A, Bernal J, Muñoz A. Identification of the mammalian homolog of the splicing regulator Suppressor-of-white-apricot as a thyroid hormone regulated gene. Mol. Brain. Res. 1999; 71: 332-340.
250. Bauer M, Heinz A, Whybrow PC. Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. Mol Psychiatry 2002; 7: 140-156.
251. Simon NM, Blacker D, Korbly NB, Sharma SG, Worthington JJ, Otto MW, Pollack MH. Hypothyroidism and hyperthyroidism in anxiety disorders revisited: new data and literature review. J Affect Disord 2002; 69: 209-217.
252. Baumgartner A, Bauer M, Hellweg R. Treatment of intractable non-rapid cycling bipolar affective disorder with high-dose thyroxine: an open clinical trial. Neuropsychopharmacology 1994; 10: 183-189.
253. Bauer M, London ED, Rasgon N, Berman SM, Frye MA, Altshuler LL, Mandelkern MA, Bramen J, Voytek B, Woods R, Mazziotta JC, Whybrow PC. Supraphysiological doses of levothyroxine alter regional cerebral metabolism and improve mood in bipolar depression. Mol Psychiatry 2005; 10: 456-469.
254. Niculescu AB, 3rd, Segal DS, Kuczenski R, Barrett T, Hauger RL, Kelsoe JR. Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach. Physiol Genomics 2000; 4: 83-91.
255. Farsetti A, Desvergne B, Hallenbeck P, Robbins J, Nikodem V. Characterization of myelin basic protein thyroid hormone response element and its function in the context of native and heterologous promoter. J. Biol. Chem. 1992; 267: 15784-15788.
256. Zou L, Hagen SC, Strait KA, Oppenheimer JH. Identification of thyroid hormone response elements in rodent Pcp-2, a developmentally regulated gene of cerebellar Purkinje cells. J. Biol. Chem. 1994; 269: 13346-13352.
257. García-Fernández LF, Urade Y, Hayaishi O, Bernal J, Muñoz A. Identification of a thyroid hormone response element in the promoter region of the rat lipocalin-type prostaglandin D synthase (beta-trace) gene. Mol. Brain Res. 1998; 55: 321-330.
258. White DM, Takeda T, DeGroot LJ, Stefansson K, Arnason BG. Beta-trace gene expression is regulated by a core promoter and a distal thyroid hormone response element. J. Biol. Chem. 1997; 272: 14387-14393.
259. Iglesias T, Caubin J, Stunnenberg HG, Zaballos A, Bernal J, Muñoz A. Thyroid hormone-dependent transcriptional repression of neural cell adhesion molecule during brain maturation. EMBO J. 1996; 15: 4307-4316.
260. Dong H, Yauk CL, Rowan-Carroll A, You SH, Zoeller RT, Lambert I, Wade MG. Identification of thyroid hormone receptor binding sites and target genes using ChIP-on-chip in developing mouse cerebellum. PLoS One 2009; 4: e4610.
261. Cuadrado A, Garcia-Fernandez LF, Imai T, Okano H, Munoz A. Regulation of tau RNA maturation by thyroid hormone is mediated by the neural RNA-binding protein musashi-1. Mol Cell Neurosci 2002; 20: 198-210.
262. Berbel P, Obregon MJ, Bernal J, Rey FE, Escobar GM. Iodine supplementation during pregnancy: a public health challenge. Trends Endocrinol Metab 2007; 18: 338-343.
263. Zimmermann MB. The Adverse Effects of Mild-to-Moderate Iodine Deficiency during Pregnancy and Childhood: A Review. Thyroid 2007; 17: 829-835.
264. Maberly GF. Iodine deficiency disorders: contemporary scientific issues. J. Nutr. 1994; 124: 1473S -1478S.
265. McCarrison R. Endemic Cretinism. In The thyroid gland in health and disease., Edition London: Bailliére, Tindall and Cox 1917; 124-147.

266. DeLong GR, Stanbury JB, Fierro-Benitez R. Neurological signs in congenital iodine-deficiency disorder (endemic cretinism). Dev. Med. Child Neurol. 1985; 27: 317-324.
267. Thilly CH, Bourdoux PP, Due DT, DeLong GR, Vanderpas JB, Ermans AM. Myxedematous cretinism: an indicator of the most severe goiter endemias. In Medeiros-Neto E, Gaitan E (eds): Frontiers in Thyroidology, Edition New York: Plenum Press 1986; 1081-1084.
268. Delange F. Neonatal screening for congenital hypothyroidism: results and perspectives. Hormone Res. 1997; 48: 51-61.
269. Trueba SS, Auge J, Mattei G, Etchevers H, Martinovic J, Czernichow P, Vekemans M, Polak M, Attie-Bitach T. PAX8, TITF1, and FOXE1 gene expression patterns during human development: new insights into human thyroid development and thyroid dysgenesis-associated malformations. J Clin Endocrinol Metab 2005; 90: 455-462.
270. Deladoey J, Vassart G, Van Vliet G. Possible non-Mendelian mechanisms of thyroid dysgenesis. Endocr Dev 2007; 10: 29-42.
271. Tiosano D, Pannain S, Vassart G, Parma J, Gershoni-Baruch R, Mandel H, Lotan R, Zaharan Y, Pery M, Weiss RE, Refetoff S, Hochberg Z. The hypothyroidism in an inbred kindred with congenital thyroid hormone and glucocorticoid deficiency is due to a mutation producing a truncated thyrotropin receptor. Thyroid 1999; 9: 887-894.
272. Dussault JH. Screening for congenital hypothyroidism. In Oppenheimer JH (ed) Thyroid Today, Edition 1980; 1-4.
273. Dubois J-M, Glorieux J, Richer F, Deal CL, Dussault JH, Van Vliet G. Outcome of severe congenital hypothyrodism: closing the developmental gap with early high dose levothyroxine treatment. J. Clin. Endocrinol. Metabol. 1996; 81: 222-227.
274. Morreale de Escobar G, Obregón MJ, Escobar del Rey F. Is neuropsychological development related to maternal hypothyroidism, or to maternal hypothyroxinemia? J. Clin. Endocrinol. Metabol. 2000; 85: 3975-3987.
275. de Zegher F, Pernasetti F, Vanhole C, Devlieger H, Van den Berghe G, Martial JA. The prenatal role of thyroid hormone evidenced by fetomaternal Pit-1 deficiency. J. Clin. Endocrinol. Metab. 1995; 80: 3127-3130.
276. Blizzard RM, Chandler RW, Landing Pettit MD, West CD. Maternal autoimmunization to thyroid as probable cause of athyreotic cretinism. N. Eng. J. Med. 1960; 263: 327-336.
277. Yasuda T, Ohnishi H, Wataki K, Minagawa M, Minamitami K, Niimi H. Outcome of a baby born from a mother with acquired juvenile hypothyroidism having undetectable thyroid hormone concentrations. J. Clin. Endocrinol. Metabol. 1999; 84: 2630-2632.
278. Man EB, Serunian SA. Thyroid function in human pregnancy. Development or retardation of 7-year-old progeny of hypothyroxinemic women. Amer. J. Obst. Gynecol. 1976; 125: 949-957.
279. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N. Engl. J. Med. 1999; 341: 549-555.
280. Momotani N, Iwama S, Momotani K. Neurodevelopment in children born to hypothyroid mothers restored to normal thyroxine (T(4)) concentration by late pregnancy in Japan: no apparent influence of maternal T(4) deficiency. J Clin Endocrinol Metab 2011; 97: 1104-1108.
281. Lazarus JH, Bestwick JP, Channon S, Paradice R, Maina A, Rees R, Chiusano E, John R, Guaraldo V, George LM, Perona M, Dall'Amico D, Parkes AB, Joomun M, Wald NJ. Antenatal thyroid screening and childhood cognitive function. N Engl J Med 2012; 366: 493-501.
282. Kooistra L, Crawford S, van Baar AL, Brouwers EP, Pop VJ. Neonatal effects of maternal

hypothyroxinemia during early pregnancy. Pediatrics 2006; 117: 161-167.
283. Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, Vulsma T, Wiersinga WM, Drexhage HA, Vader HL. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clin. Endocrinol. 1999; 50: 149-155.
284. Vermiglio F, Lo Presti VP, Moleti M, Sidoti M, Tortorella G, Scaffidi G, Castagna MG, Mattina F, Violi MA, Crisa A, Artemisia A, Trimarchi F. Attention deficit and hyperactivity disorders in the offspring of mothers exposed to mild-moderate iodine deficiency: a possible novel iodine deficiency disorder in developed countries. J Clin Endocrinol Metab 2004; 89: 6054-6060.
285. Morreale de Escobar G, Escobar del Rey F. Letter to the editor. N. Eng. J. Med. 1999; 26: 2016-2017.
286. Glinoer D. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocr. Rev. 1997; 18: 401-433.
287. Utiger RD. Maternal hypothyroidism ad fetal development. N. Eng. J. Med. 1999; 341: 601-602.
288. Berbel P, Navarro D, Auso E, Varea E, Rodriguez AE, Ballesta JJ, Salinas M, Flores E, Faura CC, de Escobar GM. Role of late maternal thyroid hormones in cerebral cortex development: an experimental model for human prematurity. Cereb Cortex 2010; 20: 1462-1475.
289. Berbel P, Mestre JL, Santamaria A, Palazon I, Franco A, Graells M, Gonzalez-Torga A, de Escobar GM. Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. Thyroid 2009; 19: 511-519.
290. Fisher DA. Thyroid function in premature infants. The hypothyroxinemia of prematurity. Clin. Perinatol. 1998; 25: 999-1014.
291. Reuss ML, Levinton A, Paneth N, Susser M. Thyroxine values from newborn screening of 919 infants born before 29 week's gestation. Am. J. Publ. Health 1997; 87: 1693-1697.
292. Morreale de Escobar G, Ares S. The hypothyroxinemia of prematurity. J. Clin. Endocrinol. Metabol. 1998; 83: 713-715.
293. Thorpe-Beeston JG, Nicolaides KH, Felton CV, Butler J, McGregor AM. Maturation of the secretion of thyroid hormone and thyroid stimulating hormone in the fetus. N. Eng. J. Med. 1991; 324: 532-536.
294. Williams FL, Simpson J, Delahunty C, Ogston SA, Bongers-Schokking JJ, Murphy N, van Toor H, Wu SY, Visser TJ, Hume R. Developmental trends in cord and postpartum serum thyroid hormones in preterm infants. J Clin Endocrinol Metab 2004; 89: 5314-5320.
295. Ares S, Quero J, Diez J, Morreale de Escobar G. Neurodevelopment of preterm infants born at 28 to 36 weeks of gestational age: the role of hypothyroxinemia and long-term outcome at 4 years. J Pediatr Endocrinol Metab 2011; 24: 897-902.
296. Den Ouden AL, Kok JH, Verkerk PH, Brand R, Verloove-Vanhorick SP. The relation between neonatal thyroxine levels ad neurodevelopmental outcome at age 5 and 9 years in a national cohort of very preterm and/or very low birth weight infants. Pediatr. Res. 1996; 39: 142-145.
297. Reuss ML, Paneth N, Pinto-Martin JA, Lorenz JM, Susser M. The relation of transient hypothyroxinemia in preterm infants to neurologic development at two years of age. N. Eng. J. Med. 1996; 334: 821-827.
298. Levinton A, Paneth N, Reuss ML, Susser M, Allred EN, Dammann O, Kubak K, Van Marter LJ, Pagano M. Hypothyroxinemia of prematurity and the risk of cerebral white matter damage. J. Pediatr. 1999; 134: 706-711.
299. La Gamma EF, van Wassenaer AG, Golombek SG, Morreale de Escobar G, Kok JH, Quero J,

Ares S, Paneth N, Fisher D. Neonatal Thyroxine Supplementation for Transient Hypothyroxinemia of Prematurity: Beneficial or Detrimental? Treat Endocrinol 2006; 5: 335-346.
300. Van Wassenaer AG, Kok JH, De Vijlder JJ, Briet JM, Smit BJ, Tamminga P, van Baar A, Dekker FW, Vulsma T. Effects of thyroxine supplementation on neurologic development in infants born at less than 30 weeks' gestation. N. Eng. J. Med. 1997; 336: 21-26.
301. Van Wassenaer AG, Kok JH, Dekker FW, Endert E, De Vijlder JJ. Thyroxine administration to infants of less than 30 weeks gestational age decreases plasma tri-iodothyronine concentrations. Eur. J. Endocrinol. 1998; 139: 508-515.
302. Gruters A. Thyroid hormone transporter defects. Endocr Dev 2007; 10: 118-126.
303. Holden KR, Zuniga OF, May MM, Su H, Molinero MR, Rogers RC, Schwartz CE. X-linked MCT8 gene mutations: characterization of the pediatric neurologic phenotype. J Child Neurol 2005; 20: 852-857.
304. Namba N, Etani Y, Kitaoka T, Nakamoto Y, Nakacho M, Bessho K, Miyoshi Y, Mushiake S, Mohri I, Arai H, Taniike M, Ozono K. Clinical phenotype and endocrinological investigations in a patient with a mutation in the MCT8 thyroid hormone transporter. Eur J Pediatr 2007.
305. Brockmann K, Dumitrescu AM, Best TT, Hanefeld F, Refetoff S. X-linked paroxysmal dyskinesia and severe global retardation caused by defective MCT8 gene. J Neurol 2005; 252: 663-666.
306. Schwartz CE, May MM, Carpenter NJ, Rogers RC, Martin J, Bialer MG, Ward J, Sanabria J, Marsa S, Lewis JA, Echeverri R, Lubs HA, Voeller K, Simensen RJ, Stevenson RE. Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. Am J Hum Genet 2005; 77: 41-53.
307. Bernal J. The significance of thyroid hormone transporters in the brain. Endocrinology 2005; 146: 1698-1700.
308. Heuer H. The importance of thyroid hormone transporters for brain development and function. Best Pract Res Clin Endocrinol Metab 2007; 21: 265-276.
309. Vaurs-Barriere C, Deville M, Sarret C, Giraud G, Des Portes V, Prats-Vinas JM, De Michele G, Dan B, Brady AF, Boespflug-Tanguy O, Touraine R. Pelizaeus-Merzbacher-Like disease presentation of MCT8 mutated male subjects. Ann Neurol 2009; 65: 114-118.
310. Dumitrescu AM, Liao XH, Weiss RE, Millen K, Refetoff S. Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (mct) 8-deficient mice. Endocrinology 2006; 147: 4036-4043.
311. Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, Raivich G, Bauer K, Heuer H. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. J Clin Invest 2007; 117: 627-635.
312. Wirth EK, Roth S, Blechschmidt C, Holter SM, Becker L, Racz I, Zimmer A, Klopstock T, Gailus-Durner V, Fuchs H, Wurst W, Naumann T, Brauer A, de Angelis MH, Kohrle J, Gruters A, Schweizer U. Neuronal 3′,3,5-triiodothyronine (T3) uptake and behavioral phenotype of mice deficient in Mct8, the neuronal T3 transporter mutated in Allan-Herndon-Dudley syndrome. J Neurosci 2009; 29: 9439-9449.
313. Liao XH, Di Cosmo C, Dumitrescu AM, Hernandez A, Van Sande J, St Germain DL, Weiss RE, Galton VA, Refetoff S. Distinct roles of deiodinases on the phenotype of Mct8 defect: a comparison of eight different mouse genotypes. Endocrinology 2011; 152: 1180-1191.
314. Di Cosmo C, Liao XH, Dumitrescu AM, Philp NJ, Weiss RE, Refetoff S. Mice deficient in MCT8 reveal a mechanism regulating thyroid hormone secretion. J Clin Invest 2010; 120: 3377-3388.
315. Trajkovic-Arsic M, Muller J, Darras VM, Groba C, Lee S, Weih D, Bauer K, Visser TJ, Heuer H. Impact of monocarboxylate transporter-8 deficiency on the hypothalamus-pituitary-thyroid axis in mice.

Endocrinology 2010; 151: 5053-5062.
316. Ito K, Uchida Y, Ohtsuki S, Aizawa S, Kawakami H, Katsukura Y, Kamiie J, Terasaki T. Quantitative membrane protein expression at the blood-brain barrier of adult and younger cynomolgus monkeys. J Pharm Sci 2011; 100: 3939-3950.
317. Refetoff S, Dumitrescu AM. Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. Best Pract Res Clin Endocrinol Metab 2007; 21: 277-305.
318. Forrest D, Golarai G, Connor J, Curran T. Genetic analysis of thyroid hormone receptors in development and disease. Rec. Prog. Horm. Res. 1996; 51: 1-22.
319. Jones I, Srinivas M, Ng L, Forrest D. The thyroid hormone receptor beta gene: structure and functions in the brain and sensory systems. Thyroid 2003; 13: 1057-1068.
320. Roberts MR, Srinivas M, Forrest D, Morreale de Escobar G, Reh TA. Making the gradient: thyroid hormone regulates cone opsin expression in the developing mouse retina. Proc Natl Acad Sci U S A 2006; 103: 6218-6223.
321. Hashimoto K, Curty FH, Borges PP, Lee CE, Abel ED, Elmquist JK, Cohen RN, Wondisford FE. An unliganded thyroid hormone receptor causes severe neurological dysfunction. Proc Natl Acad Sci U S A 2001; 98: 3998-4003.
322. Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, Henning E, Reinemund J, Gevers E, Sarri M, Downes K, Offiah A, Albanese A, Halsall D, Schwabe JW, Bain M, Lindley K, Muntoni F, Vargha-Khadem F, Dattani M, Farooqi IS, Gurnell M, Chatterjee K. A mutation in the thyroid hormone receptor alpha gene. N Engl J Med 2012; 366: 243-249.
323. van Mullem A, van Heerebeek R, Chrysis D, Visser E, Medici M, Andrikoula M, Tsatsoulis A, Peeters R, Visser TJ. Clinical phenotype and mutant TRalpha1. N Engl J Med 2012; 366: 1451-1453.
324. Vennstrom B, Mittag J, Wallis K. Severe psychomotor and metabolic damages caused by a mutant thyroid hormone receptor alpha 1 in mice: can patients with a similar mutation be found and treated? Acta Paediatr 2008; 97: 1605-1610.
325. Bernal J, Morte B. Thyroid hormone receptor activity in the absence of ligand: Physiological and developmental implications. Biochim Biophys Acta 2012.
326. Flamant F, Poguet AL, Plateroti M, Chassande O, Gauthier K, Streichenberger N, Mansouri A, Samarut J. Congenital hypothyroid Pax8(-/-) mutant mice can be rescued by inactivating the TRalpha gene. Mol Endocrinol 2002; 16: 24-32.
327. Morte B, Manzano J, Scanlan T, Vennstrom B, Bernal J. Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism. Proc Natl Acad Sci U S A 2002; 99: 3985-3989.
328. Tinnikov A, Nordstrom K, Thoren P, Kindblom JM, Malin S, Rozell B, Adams M, Rajanayagam O, Pettersson S, Ohlsson C, Chatterjee K, Vennstrom B. Retardation of post-natal development caused by a negatively acting thyroid hormone receptor alpha1. Embo J 2002; 21: 5079-5087.
329. Itoh Y, Esaki T, Kaneshige M, Suzuki H, Cook M, Sokoloff L, Cheng SY, Nunez J. Brain glucose utilization in mice with a targeted mutation in the thyroid hormone alpha or beta receptor gene. Proc Natl Acad Sci U S A 2001; 98: 9913-9918.
330. Quignodon L, Vincent S, Winter H, Samarut J, Flamant F. A point mutation in the activation function 2 domain of thyroid hormone receptor alpha1 expressed after CRE-mediated recombination partially recapitulates hypothyroidism. Mol Endocrinol 2007; 21: 2350-2360.
331. Venero C, Guadano-Ferraz A, Herrero AI, Nordstrom K, Manzano J, de Escobar GM, Bernal J, Vennstrom B. Anxiety, memory impairment, and locomotor dysfunction caused by a mutant thyroid

hormone receptor alpha1 can be ameliorated by T3 treatment. Genes Dev 2005; 19: 2152-2163.
332. Wallis K, Sjogren M, van Hogerlinden M, Silberberg G, Fisahn A, Nordstrom K, Larsson L, Westerblad H, Morreale de Escobar G, Shupliakov O, Vennstrom B. Locomotor deficiencies and aberrant development of subtype-specific GABAergic interneurons caused by an unliganded thyroid hormone receptor alpha1. J Neurosci 2008; 28: 1904-1915.
333. Liu YY, Heymann RS, Moatamed F, Schultz JJ, Sobel D, Brent GA. A mutant thyroid hormone receptor alpha antagonizes peroxisome proliferator-activated receptor alpha signaling in vivo and impairs fatty acid oxidation. Endocrinology 2007; 148: 1206-1217.
334. Liu YY, Schultz JJ, Brent GA. A thyroid hormone receptor alpha gene mutation (P398H) is associated with visceral adiposity and impaired catecholamine-stimulated lipolysis in mice. J Biol Chem 2003; 278: 38913-38920.
335. Sjögren M, Alkemade A, Mittag J, Nordström K, Katz A, Rozell B, Westerblad H, Arner A, Vennström B. Hypermetabolism in mice caused by the central action of an unliganded thyroid hormone receptor alpha1. Embo J 2007; 26: 4535-4545.
336. Chassande O. Do unliganded thyroid hormone receptors have physiological functions? J Mol Endocrinol 2003; 31: 9-20.
337. Sato Y, Buchholz DR, Paul BD, Shi YB. A role of unliganded thyroid hormone receptor in postembryonic development in Xenopus laevis. Mech Dev 2007; 124: 476-488.
338. Winter H, Braig C, Zimmermann U, Engel J, Rohbock K, Knipper M. Thyroid hormone receptor alpha1 is a critical regulator for the expression of ion channels during final differentiation of outer hair cells. Histochem Cell Biol 2007; 128: 65-75.