

A Model of the Development of the Brain as a Construct of the Thyroid System

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Thyroid hormone is essential for normal brain development. However, little is known about the molecular and cellular mechanisms that mediate thyroid hormone action on the developing brain or the developmental events selectively affected. Consequently, although a large number of environmental chemicals interfere with the thyroid system, there are few neurodevelopmental end points to recruit for toxicological studies. Therefore, my goal here is to review what is known about the relative timing of normal brain construction and thyroid system development, with special focus on the period of *in utero* development in humans and the comparable developmental period in laboratory rats. These data are presented as a timeline to aid in the identification of thyroid-sensitive end points in brain development and to highlight important data gaps. I discuss the known influence of certain synthetic chemicals on the thyroid system and include a brief review of the effects of developmental exposure to chemicals on thyroid system function. The relationship between the thyroid hormone and retinoic acid systems, as well as the thyroid hormone sensitivity of the developing cochlea, is also discussed. **Key words:** brain, development, endocrine disruptors, hearing loss, heart, retinoic acid, thyroid, xenobiotic. *Environ Health Perspect* 110(suppl 3):337–348 (2002).

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In response to the mandate of the U.S. Environmental Protection Agency (U.S. EPA) to develop a set of screens and assays to test chemicals for their possible estrogenic or other endocrine effects, a workshop was convened in June 1997 to assist the U.S. EPA in developing tests available to detect chemicals that might interfere with the thyroid system. The “Workshop on Screening Methods for Chemicals that Interfere with Thyroid Hormone Action, Function and Homeostasis” was convened at the Nicholas School of the Environment, Duke University, Durham, North Carolina by the Chemical Manufacturers Association, the U.S. EPA, and the World Wildlife Fund (1). The workshop panelists agreed that there were several assays currently in use in the medical and toxicological communities that could detect various mechanisms of thyroid system disruption and suggested using a combination of such tests to develop an assay. They also pointed out that no environmental contaminant had been identified as a thyroid hormone receptor (TR) agonist or antagonist. They recommended the use of thyroid histopathology and/or quantification of serum thyroid hormone to reduce false positives and negatives, and suggested that serum thyroid hormone concentrations could provide an initial screen in mammals. Yet, despite the counsel of the workshop participants, little progress has been made toward the development of assays to test chemicals for their impact on the thyroid system.

My purpose here is to expand upon the results of the 1997 Durham workshop with two main goals. The first goal is to produce

an inventory of data on the development of the thyroid system and concurrent development of the brain to provide a visual aid for the identification of thyroid-sensitive periods of brain maturation. The two visual aids are designed in the form of timelines based upon a literature review of scientific journals and medical reference materials: one timeline for human development from conception to birth and a second timeline for the laboratory rat from conception to postnatal day (PND) 20, the comparable developmental time period relative to humans. The second goal is to discuss what is known about how man-made chemicals can interfere with the normal production, transport, metabolism, and excretion of thyroid hormone during development, and their known impact on the thyroid system during development. Perturbation of the interaction of the thyroid and retinoic acid (RA) systems is also briefly reviewed. Finally, thyroid hormone sensitivity of the nervous system derivative of the inner ear, the cochlea, is discussed as a potential end point of thyroid hormone disruption.

The literature review presented below indicates that thyroid hormone is potentially important from the very earliest stages of development—from the mature oocyte and throughout gestation. Thyroid receptors, the proteins necessary for thyroid hormone signal transduction, have also been detected during these periods, and there is evidence of structural and functional defects in brain development when thyroid hormone is absent or in short supply. The timing of the onset of fetal thyroid function and the acquisition of

regulatory mechanisms controlling thyroid hormone action in the fetal brain indicate that thyroid hormone of maternal origin plays an important role in early developmental events in the brain. Thus, by superimposing the timing of brain development onto development of the thyroid system, it becomes clear that any environmental chemical that affects maternal thyroid status or that directly affects thyroid hormone action may exert very specific effects on the complex mechanisms underlying normal brain development.

Developing a Timeline Model of Thyroid System and Brain Development

Human Thyroid System Development

The timeline begins with a review of the basic development of the thyroid system from conception until birth in humans (Figure 1).

Thyroid gland development. Development of the human thyroid gland begins in the third week of gestation (2) (Figure 1, grid 1). On gestation day (GD) 16–17, the embryonic thyroid is visible as an outgrowth in the floor of the primitive buccal cavity (the site of the future pharynx). Additional lateral contributions to the embryonic thyroid originate from a portion of the fourth pharyngeal pouches; these lateral contributions are the origin of the C-cells, which produce calcitonin. The thyroid gland tissues merge and migrate to their final location by GD45–50. By GD70, the thyroid gland is well developed; it begins concentrating

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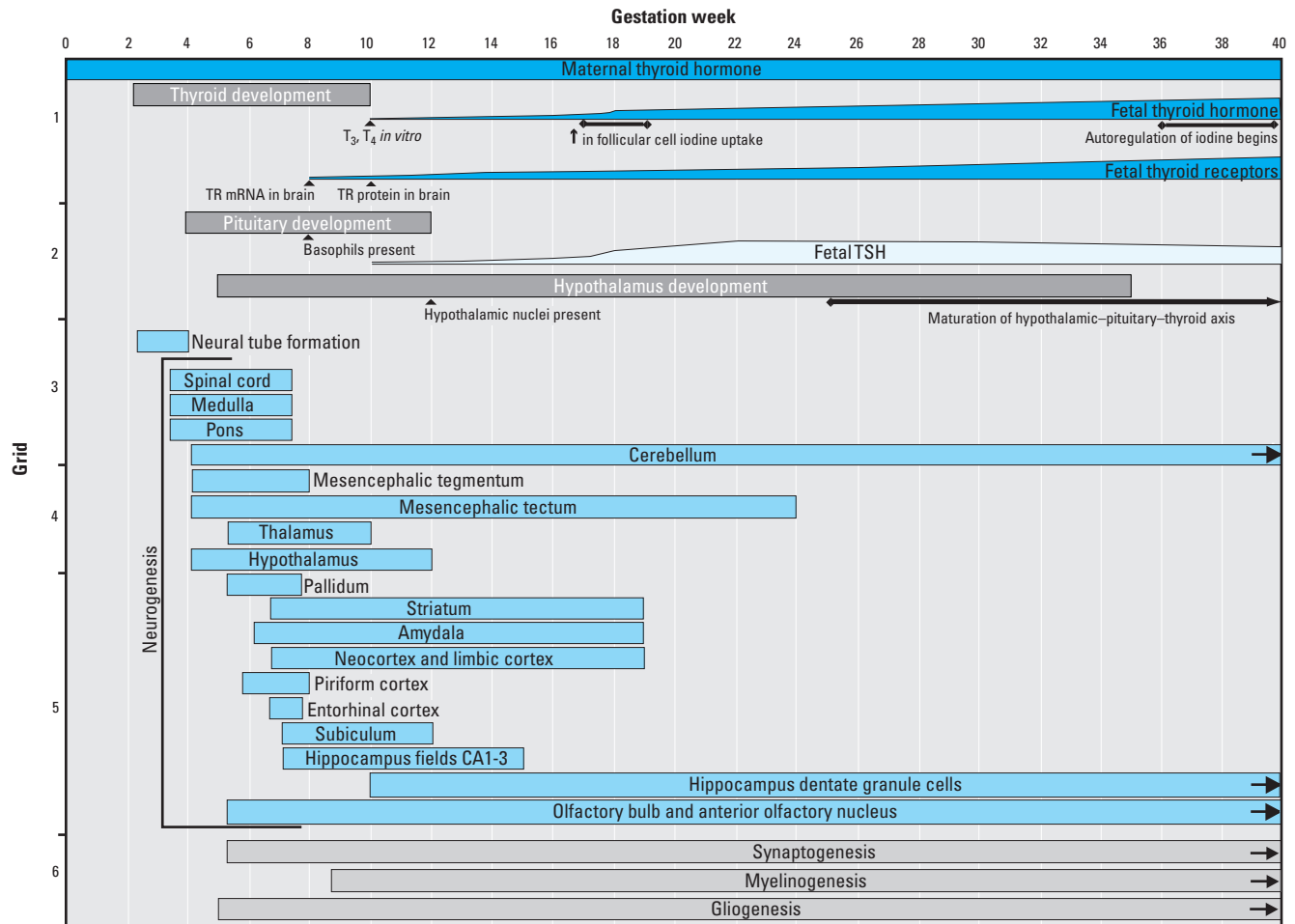


Figure 1. Timeline of human thyroid system and brain development from conception to birth. Estimation of neurogenesis adapted from Bayer et al. (49).

iodide and producing thyroid hormone at this time (3). Thyroglobulin (TBG), the precursor protein upon which thyroid hormone is produced and stored, is present as early as GD29 in thyroid follicle cells (2). As the thyroid follicle cells mature, TBG levels are detected in fetal serum by gestation week 11 and increase through gestation (4).

Maternal/fetal thyroid hormone. Thyroid hormone is produced from iodinated tyrosine residues to form 3,5,3',5'-tetraiodothyronine (thyroxine [T_4]) and 3,5,3'-triiodo-L-thyronine (T_3). T_4 is the most abundant product of the thyroid gland in circulation and is peripherally deiodinated to T_3 , the bioactive form of thyroid hormone. The bioavailability of thyroid hormone is tightly controlled by the binding of the hormone to serum-binding proteins (i.e., TBG, transthyretin, and albumin), which maintains a fixed percentage of free (not bound to serum-binding protein) thyroid hormone available to elicit thyroid action (5). During pregnancy, there is increased peripheral metabolism of free thyroid hormone; the pool of serum

protein-bound thyroid hormone also increases during this time to maintain the proper level of free hormone (6). Thus, the total (free and bound-to-serum protein) levels of maternal T_4 and T_3 increase during pregnancy to meet increased demand, while the maternal level of free T_4 remains constant (7,8) (Figure 1, grid 1).

It had long been thought that fetal exposure to maternal thyroid hormone was nonexistent or limited, because maternal T_4 is largely converted to reverse T_3 (an inactive form) in the placenta (8). However, in 1989, Vulmsa et al. (9), demonstrated that maternal T_4 did reach the fetus by observing infants with either thyroid agenesis or a total organification defect, a mutation abolishing the ability to iodinate thyroid protein. In infants with these conditions, the maternal circulation provides 35–70 nmol/L of serum T_4 (20–44% of the normal levels) as measured within the first 4 weeks of postnatal life; normal serum T_4 levels during this time period are 80–170 nmol/L serum T_4 . Maternal–embryo transfer of thyroid hormones has been detected in embryonic

coelomic fluid (total T_4 = 961 ± 193 pmol/L or 747 ± 150 pg/mL; total T_3 = 33 ± 13 pmol/L or 18.5 ± 7.3 pg/mL) and amniotic fluid (20 ± 5 pmol/L or 16.0 ± 3.8 pg/mL; total T_3 was below the detection limit of the assay) at 5 to 11 weeks of gestation (7). At term, total and free serum levels of fetal T_4 are similar to maternal values, whereas maternal serum T_3 levels (total and free) continue to be two to three times greater than fetal free T_3 levels (4). Recent studies indicate that the maternal contribution of thyroid hormone is critical for proper *in utero* brain development, as evidenced by impaired psychomotor development and visuospatial processing in offspring born to mothers with low serum levels of free T_4 (10–13).

As mentioned above, fetal thyroid hormone production begins at approximately 10 weeks of gestation in *in vitro* organ culture of human thyroid tissue (3) (Figure 1, grid 1). In an *in vivo* study, Bernal and Pekonen (14) detected T_3 , but not T_4 , in homogenized human brain tissue at 10 and 12 weeks of gestation. The tissue concentration of free T_3 is consistently higher in the

nuclear cell fraction than in the cytoplasmic fraction of 10–13 gestational week human brains, thus providing evidence for a gradient of increased free T₃ concentration in the cell nuclei versus the cytosol (15); a similar free T₃ concentration gradient from plasma to cytosol to nuclei has been reported in the adult rat brain (16).

Brain tissue levels of T₄ exceed T₃ levels at 16 weeks of gestation and beyond (14). Thorpe-Beeston et al. (4) reported increasing levels of free T₄ and T₃ in cord blood from gestation weeks 12–40. Increased thyroid hormone production is due, in part, to an increase in thyroid gland follicular cell uptake of iodide from gestation weeks 18–20 and subsequent maturation of the hypothalamic–pituitary–thyroid axis (see below). The autoregulation of iodide by the fetal thyroid gland develops during 36–40 weeks of gestation (17).

Thyroid hormone receptors. Thyroid hormone elicits its effect via binding to the TR, a nuclear transcription factor (18). The ligand-bound thyroid receptor dimerizes with a second TR and binds to a thyroid response element on the DNA to elicit gene expression. Thyroid hormone receptors are products of two genes: *c-erbA α* encoding the isoforms TR α 1 and TR α 2, and *c-erbA β* encoding the isoforms TR β 1 and TR β 2. All TR isoforms bind both T₄ and T₃, with the exception of TR α 2; TR α 2 does not bind hormone due to a mutation in its ligand-binding domain. The abundance of TR isoforms is thought to allow for the tissue specificity of thyroid hormone action. Additionally, the TR can bind with the retinoic X receptor (RXR) of the RA system, which will be described later in this article.

Thyroid hormone receptors appear before the initiation of fetal thyroid function, and they increase in association with the increase in fetal thyroid hormone production (Figure 1, grid 1). Bernal and Pekonen (14) identified TR protein in human fetal brain tissue at 10 weeks of gestation. More recently, the expression of TR α 1 and TR β 1 mRNA was detected in human whole-brain samples as early as 8 weeks of gestation (19). TR α 1 mRNA increased 8-fold from 8 through 13.0 weeks of gestation, whereas expression of TR β 1 mRNA increased at 13.9 weeks of gestation after low-level expression from 8 to 10 weeks of gestation. Similarly, Kilby et al. (20) detected the expression of all TR isoforms (TR α 1, TR α 2, TR β 1, TR β 2) in cerebral cortex samples from 10 to 16 weeks of gestation. In fetal cerebral cortex and cerebellum, only TR α 1 and TR α 2 protein was detected in the first trimester samples (11–13 weeks of gestation), while

receptor protein of all four TR isoforms was detected in the second (15–25 weeks of gestation) and third trimester (26–40 weeks of gestation) samples (20). TR mRNA and protein have been detected in the human brain at the earliest time points studied, which suggests that TR may be present even earlier in brain development. Interestingly, the mRNA of all four TR isoforms are expressed in mature human oocytes and granulosa and cumulus cells (21), thus indicating that thyroid hormone may influence development from the time of conception and early embryogenesis.

Human Hypothalamic–Pituitary–Thyroid Axis Development

Thyroid hormone production is regulated by a sensitive feedback system between the hypothalamus, pituitary, and thyroid gland. Thyrotropin-releasing hormone (TRH) is secreted from the hypothalamus, which acts upon the pituitary to stimulate the release of thyrotropin (also called thyroid-stimulating hormone [TSH]). TSH then acts upon the thyroid gland to initiate thyroid hormone production. Increased circulatory levels of thyroid hormone then provide negative feedback on the hypothalamus and pituitary to inhibit T₄ and T₃ production, thus maintaining proper levels of hormone availability.

Pituitary gland development. Although the neuroendocrine control of thyroid hormone function develops much later in fetal development, the pituitary and hypothalamic structures begin to develop within weeks of thyroid gland emergence (22,23). The anterior pituitary gland is derived from Rathke's pouch, which first appears on gestation week 4 as an outgrowth of the primitive buccal cavity (Figure 1, grid 2). By gestation week 5, the anterior pituitary is connected to a derivative of the cerebrum that will become the posterior pituitary. Cell differentiation in the pituitary is initiated by 7–8 weeks of gestation. Basophils, which include thyrotropes (thyrotropin-secreting cells) and gonadotropes (gonadotropin-secreting cells), are detected in the pituitary by 8 weeks of gestation (22), and large numbers of thyrotropes are present by 12–13 weeks of gestation (23). The development of the hypophyseal portal system, the vasculature connecting the pituitary to the median eminence, is concurrent with pituitary gland development; capillaries of the hypophyseal portal system have been detected as early as 7–8 weeks of gestation.

Thyroid-stimulating hormone. TSH is first detected in the fetal human pituitary by 10–12 weeks of gestation (22) (Figure 1, grid 2). Fetal pituitary TSH levels are relatively low until gestation weeks 16–20, after

which they increase to parturition. Fetal serum levels of TSH are first observed at 10–12 weeks of gestation. Fetal serum levels reach peak concentrations around gestation weeks 22–30, then decrease slightly until birth (22,23). The decrease in TSH before birth is thought to be, in part, in response to the onset of the negative feedback system of the maturing hypothalamic–pituitary–thyroid system.

Hypothalamus development. The hypothalamus develops from the ventral portion of the diencephalon and is visible by 5 weeks of gestation (22,23) (Figure 1, grid 2). Hypothalamic nuclei and the fibers of the supraoptic tract are detectable at 12–14 weeks of gestation. The hypothalamic nuclei and their processes continue to mature through 30–35 weeks of gestation. The hypothalamo–hypophyseal portal system, the vasculature connecting the hypothalamus with the pituitary via the median eminence, is observed in human fetuses as early as 11.5 weeks of gestation (24). This circulatory system, also referred to as the primary portal system, continues to mature through late gestation.

Thyrotropin-releasing hormone. TRH is detected in fetal whole-brain samples by 4.5 weeks of gestation (25). The levels of TRH detected this early are likely of maternal origin, as the fetal hypothalamus is just beginning to develop and maternal TRH is able to cross the placental barrier (2). TRH is detected in fetal hypothalamus extracts as early as 8–11 weeks of gestation (25,26). The hypothalamic–pituitary–thyroid axis begins to mature during the second half of gestation (22). The fetal pituitary can respond to TRH as early as 25 weeks of gestation; this is evidenced by an increase in fetal TSH secretion upon maternal administration of TRH, as measured by a cordocentesis procedure on normal pregnancies during 25–37 weeks of gestation (4).

Rat Thyroid System Development

Thyroid gland. The developing rat thyroid gland is first visible on GD9 as an endodermal thickening in the primitive buccal cavity (27) (Figure 2, grid 1). The thyroid gland is well developed and positioned in the fetal thyroid gland contains TBG and is capable of concentrating iodide. *In vitro* cultures of rat thyroid indicate its ability to synthesize thyroid hormone at least as early as GD20 (3).

Maternal and fetal/neonatal thyroid hormone. Maternal transfer of thyroid hormone to the embryo/fetus has been verified in the laboratory rat (Figure 2, grid 1). Thyroid hormone is detected in rat embryotrophoblasts as early as GD9 (total T₄ = 4.46 ± 1.04 ng/100 mg and

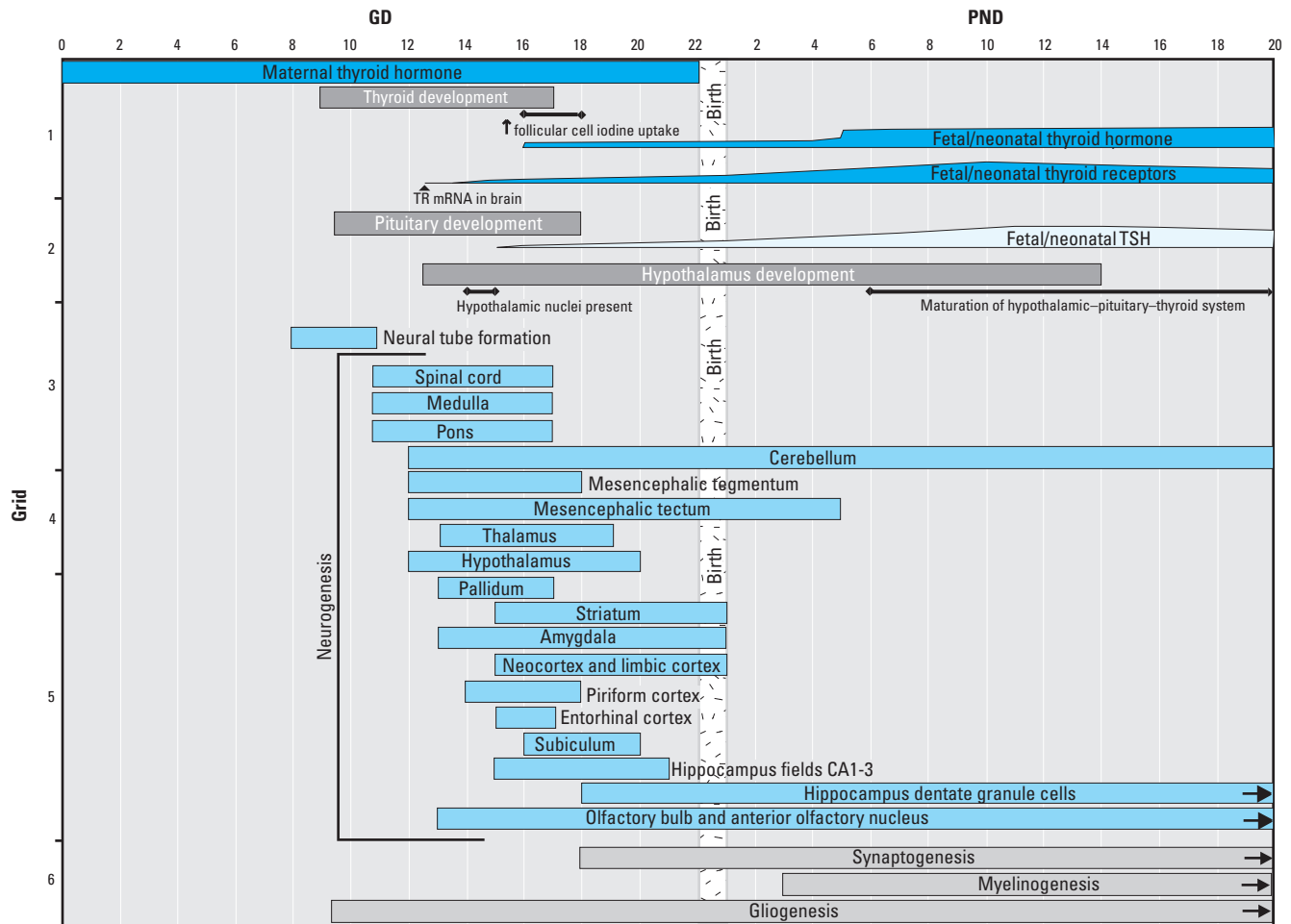


Figure 2. Timeline of rat thyroid system and brain development from conception to PND20. Conception = GD0 and birth = PND1. Timing of neurogenesis adapted from Bayer et al. (49).

total $T_3 = 0.18 \pm 0.02$ ng/100 mg (28). Woods et al. (29) reported that rat embryotrophoblasts (GD9–10) contained 21% of the T_4 and 54% of the T_3 of the maternal dose of radiolabeled T_4 or T_3 1 hr after administration. Versloot et al. (30) demonstrated that even marginal maternal iodide deficiency decreases the availability of T_4 for the fetus in the rat. Normal maternal serum T_4 levels are essential to the maintenance of fetal serum T_4 available for local conversion to T_3 (31).

The total T_4 and T_3 concentrations in rat fetuses increase dramatically from GD18 until birth because of maturation of hormone synthesis of the fetal thyroid gland (22,32) (Figure 2, grid 1). Total T_4 and T_3 tissue levels of GD18 rat fetuses measured 190 ± 38 pg/g and 33 ± 4 pg/g, respectively (28). By GD21, fetal plasma levels had reached 0.4 ± 0.03 μ g/dL total T_4 and 17 ± 4 ng/dL total T_3 . After birth, neonatal serum levels of total T_4 rise dramatically from 1.0 μ g/dL at PND5 to a peak level of 6 μ g/dL at PND17 (33). Neonatal serum levels of total T_3 rise in

parallel to total T_4 with a peak serum value of 108 ng/dL reached on PND28. Free serum T_4 and T_3 levels follow a pattern similar to the total serum values (22). The serum levels of T_4 and T_3 reach adult values at approximately PND40.

Fetal/neonatal thyroid hormone receptors. Fetal TRs are present early in fetal rat brain development (34). TR β 1 and TR β 2 isoforms were detected as early as GD12.5 in the rat otic vesicle in the fetal brain, the portion of the embryonic inner ear that develops into the cochlea (35) (Figure 2, grid 1). Falcone et al. (36) detected TR α 1, TR α 2, and TR β 1 protein at GD14 in rat brains, particularly in regions of neuronal and glial cell nuclei. Levels of TR in the fetal brain increase 3-fold from GD14 to GD16–17, then remain steady until birth (34). Following birth, TR mRNA and protein levels increase through the second week of postnatal life, reaching peak mRNA concentrations on PND10 and protein concentrations on PND15 (37,38).

There is evidence that the TR α and TR β isoforms are selectively expressed in different brain regions during development

and may therefore mediate different functions. For example, Bradley et al. (39) found that the TR β 1 mRNA is selectively expressed in the ventricular zone of the early cortex where cells undergo proliferation and early fate specification. However, as these cells leave the cell cycle and begin to differentiate, they migrate away from the ventricular zone, cease expressing TR β 1, and begin expressing TR α 1. Likewise, Strait et al. (40) observed a selective pattern of TR β 1 and TR α 2 expression in cerebellar sections of neonatal (PND4) and adult (PND60) rats. TR β 1 protein was strongly localized to the nuclei of Purkinje cells and weakly present in the nuclei of granule cells, whereas TR α 2 was detected only in the nuclei of granule cells. Neither TR isoform was detected in the neuronal axons or glial cells. In contrast, Leonard et al. (41) concluded that TR α 2 is selectively expressed in astrocytes, but not neuronal cells. Although studies using transgenic mice and targeted deletion/mutations have helped elucidate some of the physiological functions of specific TR isoforms (42),

distinct roles for the TR isoforms in specific neurodevelopmental events have yet to be identified.

Rat Hypothalamus–Pituitary–Thyroid Axis Development

Pituitary development. The first sign of rat anterior pituitary development is the formation of Rathke's pouch, which appears on GD9.5 (27) (Figure 2, grid 2). By GD16, the pituitary is well developed and secretory granules are present (22). The number of granule cells increases from GD18 through the end of the first postnatal week. The posterior pituitary is well developed by GD18. The pituitary portal system develops in concert with the pituitary gland and is well developed by GD17.

Thyroid-stimulating hormone. Pituitary TSH mRNA expression begins on GD15 (43), while TSH protein levels are first reported at GD17 measuring 24.8 ± 2 ng/pituitary (44) (Figure 2, grid 2). Fetal pituitary levels climb to 162.0 ± 9.64 μ g/pituitary for males and 194.5 ± 9.14 μ g/pituitary for females. Serum levels of TSH are 20 ng/100 μ L at birth and increase to maximum postnatal serum levels by PND7–8 (40 ng/100 μ L TSH) (22). Pituitary levels of TSH increase from 170 ng/ μ g protein at birth to approximately 1 μ g/ μ g protein by PND10–12. The levels of pituitary and serum TSH slowly decrease from PND14–16 until reaching adult levels at PND40 (33).

Hypothalamus development. The developing hypothalamus is first visible on GD12.5 as the diencephalon differentiates into the hypothalamus, epithalamus (which comprises the pineal gland), and the thalamus (27) (Figure 2, grid 2). By GD14–15, hypothalamic nuclei are present and there is continued ingrowth of axons into the median eminence (22). The hypothalamus and median eminence are nearly morphologically complete by the third postnatal week. The vasculature of the hypothalamo–hypophyseal portal system is known to reach the median eminence late in the first postnatal week, with continued development of the portal system occurring through postnatal weeks 5–6.

Thyrotropin-releasing hormone. TRH mRNA can be detected as early as GD14 in neurons of the rat fetal hypothalamus (45). By GD15, TRH mRNA is detected in the developing paraventricular nuclei of the hypothalamus. At birth, TRH mRNA was detected in all areas of the brain known to express it in adulthood. Adult TRH mRNA expression patterns are present by PND22. TRH is produced in low levels (6.0 ± 0.5 pg/mg) in the rat hypothalamus as early as GD16 and increases approximately 3-fold

by GD20 (46). TRH levels increase to adult levels by PND17–29, then decrease transiently between PND31–41; adult levels are once again reached at PND50 (33,44). Although TRH is present in the hypothalamus in late gestation, it does not appear to influence the hypothalamic–pituitary–thyroid axis until the second week of postnatal life (22,47).

Mammalian Brain Development (Human and Rat)

Central nervous system embryogenesis begins with formation of the neural tube. This process begins around 2 weeks of gestation in humans (GD8–9 in rats), with the neural plate developing from ectoderm as directed by the notochord (27,48) (Figure 1, grid 3 human; Figure 2, grid 3 rat). As the neural plate invaginates and begins to fuse, neural crest cells are derived from the ectoderm at the interface closing neural tube and skin ectoderm. The neural crest cells are progenitors of several components of the nervous system, including the sensory ganglia of the spinal and cranial nerves, the Schwann cells (neuroglial cells covering the peripheral nerves), the meninges (connective tissue covering of the brain), and other derivatives. The neural tube closes in a caudal to rostral pattern with hindbrain (rhombencephalon) regions forming before forebrain (telencephalon) regions. In humans, the neural tube formation begins at approximately GD18 (rats ~GD9–9.5) and is complete by GD26–28 (rats GD10.5–11) (48).

Bayer et al. (49) estimated the timing of human fetal brain neurogenesis based upon extensive histological observation of rat fetal and neonatal brain development compared with anatomical drawings and photographs of human fetal brain development. The initiation of neurogenesis in the developing brain proceeds in a caudal to rostral direction beginning with the spinal cord and the hindbrain derivatives, including the myelencephalon (medulla) and metencephalon (pons and cerebellum) (Figure 1, grid 3 human; Figure 2, grid 3 rat). Neurogenesis subsequently begins in the mesencephalon (tectum and tegmentum) and diencephalon (thalamus and hypothalamus) (Figure 1, grid 4 human; Figure 2, grid 4 rat). The telencephalon is the last of the brain regions to begin to develop; it encompasses the cerebral structures, including the basal ganglia (striatum and pallidum), amygdala, and cerebral cortex (neocortex, limbic and piriform cortices, hippocampal regions, and the olfactory bulb) (Figure 1, grid 5 human; Figure 2, grid 5 rat). Neurogenesis in the cerebellum, hippocampus dentate granule

cells, and the olfactory bulb continue into postnatal life in humans and rats (49). Recent studies in rodents and primates suggest that regions of the hippocampus and olfactory bulb continue to undergo neurogenesis well into adulthood (50,51).

The initiation of migration of neurons is concurrent with the period of neurogenesis and is usually completed within days in the rat and within weeks in humans (49). Differentiation is a process that generally begins after a brief pause following migration (49); however, cell fate determination may begin as early as migration (48). In humans, synaptogenesis is reported to begin as early as gestation month 2 and continues into postnatal life, with a peak in the first year of life (52) (Figure 1, grid 6). In rats, synapse formation is detected as early as GD18 in the substantia nigra (53), with the majority of synapse formation occurring after birth (54,55) (Figure 2, grid 6). Myelin production first appears around gestation month 5 in humans and continues well into postnatal life (52) (Figure 1, grid 6). Myelin protein mRNA is clearly expressed in cranial nerve VIII (56) by PND2, and in the cerebellum and mesencephalon by PND10, followed by expression in more rostral brain regions later in rat development (57) (Figure 2, grid 6).

Thyroid Hormone Influence on Brain Development

Thyroid hormone is essential for brain development (37, 58–62); the following section is a brief summary of some of thyroid hormones' effects on neuronal proliferation, migration, synaptogenesis and myelination. For example, in the cerebellum, thyroid hormone plays an important role in granule cell proliferation and survival. Granule cell proliferation, as measured by 3 H-thymidine, is enhanced by thyroid hormone and suppressed by hypothyroidism (63). In addition, granule cell apoptosis, as measured by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling, was increased 8-fold in hypothyroid animals (64). Direct effects of thyroid hormone on the expression of genes encoding growth factors and antiapoptotic genes may mediate these effects. Neveu and Arenas (65) found that hypothyroidism increased the abundance of mRNAs encoding nerve growth factor and neurotrophin 4/5 and severely reduced the expression of the mRNAs encoding neurotrophin 3 and brain-derived neurotrophic factor in the cerebellum. However, there were no effects of thyroid hormone on levels of the full-length tyrosine kinase B and C receptor mRNAs. In addition, Muller et al. (66) found that

Bcl-2 (B-cell lymphoma 2 protooncogene) administration prevented the increased apoptosis in the cerebellum induced by hypothyroidism.

Thyroid hormone availability influences neuronal migration, as evidenced by decreased whole-brain weight and densely packed neurons in the hypothyroid rat cerebellum (63,67). Many genes involved in the process of neuronal migration are sensitive to thyroid hormone (62). For example, Alvarez-Dolado et al. (68) reported that thyroid hormone increases mRNA expression and translation of *reelin*, which encodes a large extracellular protein. Conversely, hypothyroid conditions significantly reduce the abundance of *reelin*-containing cells and the intensity of signal from each cell. Neural migration is also influenced by thyroid hormone effects on astrocytes, a glial cell type. Astrocytes secrete laminin, a key guidance signal for the migration of neurons (69). In the developing rat cerebellum, thyroid hormone deficiency delays the expression of laminin, and total laminin concentration is 35% less intense than in controls (70).

Synaptogenesis is negatively affected in the hypothyroid brain due to the decrease in axonal length and branching of neurons, thus reducing neuronal connections. Hypothyroidism decreases neural axonal density and growth in the cerebral cortex (71) and reduces the dendrite branching of Purkinje cells in the cerebellum (72), pyramidal cells in the cerebral cortex (73), and cortical neurons of the corpus callosum (74). Rami and Rabie (75) report a delay in hippocampal synaptogenesis under hypothyroid conditions, based on measurements of synaptophysin, a marker protein of mature synaptic vesicles. In the cerebellum, mature granule cells in the internal granular layer express a synaptotagmin-related gene 1 (*Srg1*) (76). Thyroid hormone upregulates *Srg1* mRNA, whereas hypothyroidism decreases its expression. Finally, a reduction in myelination is also a common phenotype of the hypothyroid neonatal brain. The decrease in myelination is due, in part, to the decrease in length and branching of neural outgrowths as previously described. Thyroid hormone also influences myelination by stimulating the differentiation of oligodendrocytes, which are the glial cells responsible for the production of the myelin proteins (77). Ibarrola and Rodríguez-Peña (57) report that hypothyroidism causes delayed expression of four major myelin protein genes: myelin basic protein (MBP), proteolipid protein (PLP), 2':3'-cyclic nucleotide 3'-phosphodiesterase, and myelin-associated glycoprotein; the delayed expression of

such genes leads to an overall decrease in myelin protein production. In contrast, hyperthyroidism increases the MBP and PLP mRNA, and this effect is likely due to an increased abundance of mature oligodendrocytes (77).

Gliogenesis and its responsiveness to thyroid hormone has most often focused on the development and maturation of the astrocytes and oligodendrocytes, which develop well after the bulk of neurogenesis is complete (48,77). However, radial glial and microglial cells are present much earlier in brain development and are also influenced by thyroid hormone. Radial cells provide a framework for neuronal migration (78,79) and are the precursor cell line to astroglial cells (80). In mice, radial glial cells are present in the developing neural tube as early as GD9.5 (81) and by estimated GD11 in rats (27). Microglial cells are macrophage cells, which phagocytize dead cells and cellular debris following apoptosis during brain development. In mice, microglia progenitor cells are derived from the yolk sac and are present in future brain tissue by GD8 and mature microglial are detected by GD13 (82); in rat the estimated time points are GD9.5 and GD14, respectively (27) (Figure 2, grid 6). Radial and microglia are also present very early in human gestation (52,83) (Figure 1, grid 6). Prenatal hypothyroidism inhibits the maturation of radial glial cells in the CA1 region of GD21 rat hippocampus, as measured by the abundance of glial fibrillary acidic protein (GFAP) (84). A deficiency of GFAP during development inhibits the branching necessary for astrocyte-neuronal connections and decreases the long-term potentiation capacity, a factor essential to learning and memory (85). Microglial cells express the TR α 1 and TR β 1 isoforms and are influenced by thyroid hormone (86). Microglial cell density and process formation are depressed in the parietal cortex of neonatal rats exposed to hypothyroid conditions from GD16 through lactation (86). In contrast, hyperthyroidism induced by T₃ injections to euthyroid and prenatally hypothyroid rat pups increased microglial cell survival and branching of the developing axonal fiber tracts of the corpus callosum. Although Lima et al. (86) identify the first postnatal week in rats as a critical window for thyroid hormone influence, they suggest that maternal thyroid hormone could be influencing the early development of fetal microglial cells. The fact that both radial glial and microglial cells are negatively affected by hypothyroid conditions indicates that thyroid hormone may influence very early stages of brain development.

The complexity of development within each brain region suggests that investigations of thyroid hormone influence on the brain should be conducted with as much specificity to region as possible. For example, different cell populations within each area of the brain often have their own unique timeline for birth and maturation (49,52). Neurogenesis of the Purkinje and granule cells populations occurs at different times in the cerebellum; Purkinje cells emerge between GD13–15 in the rat (human gestation weeks 5.3–7.0), whereas the granule cells do not begin to appear until PND4 in the rat (human gestation week 24.0) (49). Similarly, the peak proliferation of granule cells in the hippocampus occurs after birth, whereas maximal neurogenesis of the hippocampal CA3 neurons occurs between GD17 and GD20 (49). Different regions of the brain also have particular developmental schedules. As demonstrated in Figures 1 and 2, neurogenesis is initiated in hindbrain derivatives (i.e., medulla and pons) well before it begins in forebrain derivatives (i.e., neocortex and hippocampus) in both humans and rats (49). Similarly, Herschkowitz et al. (52) reviewed the timing of brain development in humans and found the onset of myelination occurred at specific times for the various brain areas. For example, myelination of the spinal nerve roots begins around gestation month 5, whereas myelination of the auditory cortex does not begin until gestation week 9. Likewise, myelinogenesis in the rat brain occurs in a regional pattern (87). Myelination of the vestibulo-cochlear nerve (cranial nerve VIII) is evident by PND2 (56), whereas onset of myelin protein gene expression in the cerebellum and cerebral cortex occurs on PND10 and PND20, respectively (57).

Interaction of Retinoic Acid and Thyroid Systems during Brain Development

The interaction of the vitamin A and thyroid systems is important for eliciting thyroid hormone action (88). Vitamin A (retinol) is obtained from the diet and passes through two enzymatic conversions to derive the bioactive metabolite RA. Like TR, RA receptors are members of the steroid/retinol/vitamin D/thyroid hormone nuclear receptor superfamily. There are two classes of RA receptors: the retinoic acid receptor (RAR), which binds most naturally occurring RAs, and RXR, which binds only 9-*cis*-RA. These RA receptor isoforms elicit RA action by binding as homodimers or heterodimers with each other. Thyroid receptors can heterodimerize with both classes of RA receptors (18).

However, TR preferentially heterodimerizes with the RXR, which often results in more effective activation of the corresponding thyroid or RA response element than a TR or RXR homodimer (88). In the rat, TSH β gene expression is regulated by T₃ and RA via TR-RXR and RAR-RXR heterodimers, respectively (89). *In vitro* studies of rat optic nerve oligodendrocyte precursor cells determined that RA as well as thyroid hormone plays a role in promoting the differentiation of these cells into mature oligodendrocytes cultures, possibly through influence on the activity of transcription factor activator protein 1 (90). Koibuchi and Chin (91) reported that thyroid hormone accelerated the initiation of RA orphan receptor α expression in developing rat Purkinje cells in the hypothyroid rat cerebellum, suggesting that thyroid hormone may contribute to the Purkinje cell differentiation via interaction with the RA system.

Xenobiotic Interference with the Thyroid System

As mentioned in my introduction, the panelists at the Duke University workshop agreed that there were assays currently in use that could detect changes in thyroid hormone action and gland function (1). They identified a number of assays that could be used to screen chemicals for their potential to interfere with the thyroid system but emphasized that none of the assays were adequate as a first-tier screen. The assays were as follows: *a*) a TRH challenge, which tests the integrity of the hypothalamic-pituitary-thyroid axis by measuring TSH production following the administration of TRH; *b*) the use of a perchlorate discharge test to detect chemicals that inhibit the uptake of iodide by the thyroid gland; *c*) a quantification of thyroid peroxidase, the key enzyme in thyroid hormone production that assures the oxidation of iodide, the incorporation of iodide into the tyrosine residues on TBG, and the coupling of the di- and triiodotyrosyl residues on TBG to form thyroid hormone; *d*) a series of thyroid hormone competitive binding assays to quantify maternal serum-binding proteins, which can predict changes in fetal thyroid hormone concentrations; *e*) a measure of iodothyronine deiodinase activity of the three tissue specific enzymes (type I, II, III proteins) that facilitate the removal of iodine from thyroid hormone; and *f*) a measure of the rate of thyroid hormone glucuronidation using an *in vivo* exposure paradigm followed by *ex vivo* examination using hepatic microsomes. Glucuronidation of thyroid hormone, which is facilitated by the enzyme uridine diphosphate-glucuronyl transferase (UDP-GT), produces an

inactive, excretable form of the hormone; under normal conditions, glucuronidation helps to maintain the proper free thyroid hormone levels.

Indeed, synthetic chemicals can disrupt nearly every step in the production and metabolism of thyroid hormone (92,93) (Table 1). Chemical interference with uptake of iodide by the thyroid gland and, more specifically with the sodium/iodide symporter (which facilitates the iodide uptake), can result in a decrease in the circulating levels of T₄/T₃ (94,95). Chemical exposure can also lead to a decrease in serum protein-bound iodide levels, perhaps largely due to inhibition of the thyroid peroxidase enzyme, which disrupts the normal production of thyroid hormone (96–98). The displacement of T₄/T₃ from the transport proteins (TBG, transthyretin, and albumin) may result in decreased ability of thyroid hormone to reach its target tissue and may facilitate the transport of the chemicals into the fetus (92,99). Chemical disruption of the T₄/T₃ metabolism can influence deiodinase, glucuronidase, and sulfatase activity, and may ultimately result in increased biliary elimination of T₄/T₃. Inhibition of the deiodinase enzymes can result in a decrease in T₃ available to elicit thyroid action at the tissue level (100). Conversely, deiodinase activity may increase in response to chemical exposure, either as a direct effect or in response to increased clearance of T₄/T₃ by the chemical stimulation of glucuronidase or sulfatase enzymes (101,102). Brucker-Davis (93) suggests that such increases in the metabolism and clearance of T₃ could result in a goiter as the thyroid gland increases production to maintain proper hormone levels. The list in Table 1 of 116 chemicals capable of disrupting normal thyroid hormone production, transport, and metabolism is by no means exhaustive; further discussion of the effects of disruption of these processes can be found in reviews by Brouwer et al. (92) and Brucker-Davis (93). There are many more chemicals that have effects on TSH and T₄/T₃ levels, and thyroid histopathology for which no mechanism has been tested (93). It is unlikely that these chemicals are working as T₄/T₃ agonists or antagonists at the level of TR binding, as no chemical tested thus far has demonstrated high affinity binding to the mammalian TR. (103,104).

Relatively few studies have evaluated the mechanism of action of thyroid-disrupting chemicals in the fetal/neonatal organism. Darnerud et al. (105) reported that developmental exposure to 4-OH-3,5,3',4'-tetrachlorobiphenyl, a major metabolite of polychlorinated biphenyl

(PCB) congener 3,3',4,4'-tetrachlorobiphenyl (PCB 77), binds to fetal and maternal transthyretin in mice on GD17; significant decrease in fetal T₄ (free and total) was reported. Aminotriazole inhibited the catabolism of T₄ to T₃ in renal primary cell cultures from 4 to 5 months of gestation in human fetuses, indicating an interference with type 1 iodothyronine deiodinase function in the kidney (106). *In utero* exposure to PCB congener 3,3',4,4',5,5'-hexachlorobiphenyl alone or in combination with PCB 77 increased type II deiodinase activity in whole-brain homogenates from fetal (GD20) and neonatal (PND7 and PND21) rats; total T₄ levels in plasma were decreased by both treatments (107). UDP-GT activity was increased in PND21 rat weanlings exposed to the PCB congeners PCB 77, PCB 126 (3,3',4,4',5-pentachlorobiphenyl), or TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) on GD10 (108). The increase in UDP-GT activity was seen in the near absence of significant decreases in T₄ concentration on PND21 (108). Gestational exposure to Aroclor 1254 depressed UDP-GT activity in GD20 rat fetuses, while increasing the enzyme in PND21 rats (109). The total and free T₄ levels in GD20 fetuses were significantly suppressed by both levels of Aroclor 1254 exposure during development, whereas the total T₄ and total T₃ were significantly depressed on PND21 only by the highest dose of Aroclor 1254 (109). The ability of man-made chemicals to disrupt the thyroid system strongly suggests the possibility of chemical perturbation of thyroid-sensitive brain development (92,110,111).

Chemical perturbation also influences the interaction of the thyroid hormone and RA systems. In young adult rats, RA is displaced from its serum transport protein, retinol binding protein, and the transthyretin-retinol binding protein complex by PCB 77 (112). Similar effects by PCB 77 on binding to transport proteins and serum levels of T₄ have been reported (99,105). Subchronic exposure to 2,2',3,3',4,4'-hexachlorobiphenyl decreased kidney and liver levels of vitamin A and significantly lowered frontal cortex dopamine levels in rats dosed for 4 weeks after weaning (113). Reduced follicle size and papillary proliferation of the thyroid epithelium were noted in all treatment levels. Hallgren et al. (114) reported that polybrominated diphenyl ether (PBDE) and Bromokal (a PBDE mixture) given orally to adult rats and mice significantly depressed serum T₄. Bromokal also reduced vitamin A levels in the liver and induced UDP-GT activity at the highest

Table 1. Synthetic chemicals that interfere with the production, transport, and metabolism of thyroid hormone.

Thyroid mechanism and interfering chemical		
Uptake of iodide by thyroid gland		
2,4-D (137)	Mercuric chloride (153)	1,4-Tetrachlorophenol (99,170)
3-Amino-1,2,4-triazole (138,139)	<i>o,p'</i> -DDD (161,162)	PCB-77 (99,105,169)
Aldrin (140)	Hexadrin (147)	Trichloroacetic acid (99)
Amitrole (141,142)		Trichlorobenzene (99)
Aroclor 1254 (143,145)	Thyroid peroxidase action—general information	2,3,4-Trichlorophenol (170)
1,2-Dihydroxybenzene (catechol) (146)	Amitrole (141)	2,4,5-Trichlorophenol (99,169,170)
4-Chlororesorcinol (146)	Ammonia (163)	2,4,6-Trichlorophenol (99,170)
Clofentazine (141)	Ethylene thiourea (141)	2,4,5-Trichlorophenoxyacetic acid methyl ester (99)
<i>o</i> -Cresol (146)	Fipronil (141)	
<i>p</i> -Cresol (146)	Mancozeb (141)	Binding to albumin
Cythion (96,147)	4,4'-Methylenedianiline (141)	Pentachlorophenol (169)
1,3-Dihydroxynaphthalene (146)	Thiocyanate (141)	
1,5-Dihydroxynaphthalene (146)	Thyroid peroxidase action—oxidation of iodide	Catabolism of T₄ or T₃: type I or II 5'-deiodinase
2,3-Dihydroxynaphthalene (146)	Aminotriazole (97,164)	3,3',4,4',5,5'-Hexachlorobiphenyl (107)
2,7-Dihydroxynaphthalene (146)	Ammonia (163)	3-Methylcholanthrene (171,172)
2,4-Dihydroxybenzaldehyde (146)	Cadmium chloride (163,165)	Aminotriazole (106)
2,4-Dihydroxybenzoic acid (146)	Endosulfan (166)	Amiodarone (94,172)
Ethiozin (141)	Ethylene thiourea (98)	Aroclor 1254 (109)
Ethylene thiourea (141,148)	1,2,3,4,5,6-Hexachlorocyclohexane (lindane) (167)	Cadmium chloride (173)
Fipronil (141)	Malathion (167)	Diphenylthiohydantoin (141,172)
Hexachlorobenzene (149,150)	Mancozeb (152)	Dimethoate (100,174)
Hexadrin (147)	Mercury chloride (165)	Fenvalerate (175,176)
4-Hexylresorcinol (146)	Methamizole (97)	Hexachlorobenzene (102)
1,3,4-Trihydroxybenzene (hydroxyquinol) (146)	Polybrominated biphenyls (158)	Lead (177)
Hydroxyquinol triacetate (146)	Thiourea (166)	Phenobarbital (172)
Lead (151)		Propylthiouracil (172)
Mancozeb (152)	Thyroid peroxidase action—iodination of tyrosine	PCB 77 (107,171)
Mercuric chloride (153,154)	Polybrominated biphenyls (158)	TCDD (171,178)
3-Methylcholanthrene (143,155)		Glucuronidation of T₄/T₃
Methylmercuric chloride (154)	Binding to thyroglobulin	Acetochlor (141)
Methylparathion (156)	<i>o,p'</i> -DDD (161)	Aroclor 1254 (109,143–145,179)
2-Methylresorcinol (146)	Pentachlorophenol (168)	3,4-Benzopyrene (180)
Mull-Soy (157)		Clofentazine (141)
Nabam (140)	Binding to transthyretin	Clofibrate (141)
5-Methylresorcinol (orcinol) (146)	Bromoxynil (3,5-bibromo-4-hydroxybenzotrinitril) (99)	DDT (144)
Pendimethalin (141)	4-(Chloro- <i>o</i> -tolylxy) acetic acid (99)	Fenbuconazole (141)
Pentachloronitrobenzene (141)	4-(4-Chloro-2-methylphenoxy) butyric acid (99)	3,3',4,4',5,5'-Hexabromobiphenyl (101)
Phenobarbital (143)	Chlorophenol (99,169)	Hexachlorobenzene (102,183)
Phenol (146)	Chloroxuron (99)	2,3,3',4,4',5'-Hexachlorobiphenyl (182)
1,3,5-Trihydroxybenzene (phloroglucinol) (146)	2,4-D (99)	3,3',4,4',5,5'-Hexachlorobiphenyl (107)
Polybrominated biphenyls (158)	2,4-Dichlorophenoxybutyric acid (99)	3-Methylcholanthrene (141,143,155,171,179)
Pregnenolone-16 α -carbonitrile (143)	Dioxyolphthalate (99)	Pendimethalin (141)
Propylthiouracil (139,158)	<i>o,p'</i> -DDD (99)	PCB 126 (108,182)
1,2,3-Trihydroxybenzene (pyrogallol) (146)	<i>p,p'</i> -DDD (99)	Phenobarbital (141,143,172,180,181,183)
Pyrimethanil (141)	2,3-Dichlorophenol (99,169)	Polybrominated biphenyls (184)
1,3-Dihydroxybenzene (resorcinol) (146)	2,4-Dichlorophenol (99)	PCBs (141)
<i>o</i> -Hydroxybenzyl alcohol (saligenin) (146)	2,6-Dichlorophenol (99,169,170)	Pregnenolone-16 α -carbonitrile (141,143,179)
Selenium (151)	2-(2,4-Dichlorophenoxy) propionic acid [dichloroprop] (99)	Promadiazine (141)
Thiocyanate (141)	1,1,1-Trichloro-2,2-bis (chlorophenol) ethanol [difocol] (99)	Pyrimethanil (141)
	2,4-Dinitrophenol (99)	PCB 77 (108,171)
	2,4-Dinitro-6-methylphenol (99)	TCDD (108,141,178,182)
	Ethyl-bromophos (99)	Thiazopyr (141)
	Ethyl-parathion (99)	
	2-(2,4,5-Trichlorophenoxy) propionic acid [fenoprop] (99)	Catabolism and biliary elimination of T₄/T₃ in the liver
	Hexachlorobenzene (99)	Aroclor 1254 (144,145)
	Hexachlorophene (99,169)	3,4-Benzopyrene (180)
	2-Hydroxybiphenyl (99)	DDT (144)
	4-Hydroxybiphenyl (99,169)	Hexachlorobenzene (102)
	Lindane (99)	3-Methylcholanthrene (155)
	Linuron (99)	Phenobarbital (180,183)
	Malathion (99)	Polybrominated biphenyls (184)
	Pentachlorophenol (99,169,170)	
	Phenol (169)	
	Pyrogallol (99)	
	2,4,5-Trichlorophenoxyacetic acid (99)	

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane.

dose in the rats; similar results were found for 2,3,3',4,4'-pentachlorobiphenyl and Aroclor 1254 (114). Further research is needed to better understand how disruption of the vitamin A system may influence the thyroid system and brain development.

Thyroid Sensitivity of Nervous System Derivatives

There are several nervous system derivatives influenced by thyroid hormone. Thyroid hormone receptor $\beta 2$ isoform has been identified in the outer nuclear layer of the mouse retina, which contains the developing photoreceptors (115) and is thought to play a role in committing the photoreceptor progenitor cells to becoming green cone photoreceptor cells. The pacemaker cells of the mammalian heart contain TR $\alpha 1$ (42,116) and T $_3$ may influence pacemaker activity by increasing the ion (Na $^+$ -Ca $^{2+}$) activity (117). The following section will discuss the thyroid-sensitive nature of the development and function of the cochlea. Impaired function of the cochlea as well as of the eye and heart have been reported in laboratory studies as a result of thyroid perturbation during development and could be useful end points in detecting thyroid disruption.

Cochlear Development

Hearing loss is commonly associated with thyroid disorders in humans, including congenital hypothyroidism (118,119), iodide deficiency (120), resistance to thyroid hormone (121), and Pendred syndrome (122). Likewise, deafness is reported in the *hyt/hyt* mouse model with congenital hypothyroidism (123). TR $\beta 1$ and TR $\beta 2$ mRNA are expressed in the rat fetus as early as GD12.5 in the region of the otic vesicle that gives rise to the cochlea (35). Auditory function is mediated specifically by TR β , as evidenced by hearing loss in mice lacking a functional TR β , whereas mice deficient in TR α have normal hearing (124,125). TR β is necessary for development of cochlear function; mice deficient in TR β exhibit delayed expression of a key potassium conductance necessary for the maturation of the cochlear inner hair cells (125). Thyroid hormones acting through TR β also influence hearing by initiating myelinogenesis of the cochlea and vestibulocochlear nerve (cranial nerve VIII) prior to the onset of hearing (56). In rats, the critical periods of thyroid-sensitive ear development extend from at least GD18 (the onset of fetal thyroid gland function) through PND18 (the period of outer hair cell synaptogenesis in the cochlea) (126–128). Brucker-Davis et al. (121) estimate the critical period of thyroid-sensitive

cochlear development in humans extends from the close of the first trimester through the first month of life.

Hypothyroidism caused by developmental exposure to thyroid-disrupting chemicals can also cause hearing loss. Adult rats developmentally exposed to propylthiouracil (129), methimazole (130), or Aroclor 1254 (131) have significantly elevated auditory thresholds (i.e., less sensitive hearing) relative to controls. Hearing impairment caused by developmental exposure to Aroclor 1254 was identified as cochlear and/or auditory nerve dysfunction (132). It was due, in part, to a decrease in the abundance of outer hair cells in the upper middle turn (responsible for detection of lower frequency sounds) as well as the apical turn of the cochlea in rats (133). In a subsequent experiment, postnatal administration of T $_4$ partially blocked low-frequency hearing loss in rats developmentally exposed to Aroclor 1254 (134). With regard to human exposure, Chao et al. (135) compared children in Yucheng, Taiwan, who were born to women exposed to PCB- and polychlorinated dibenzofuran-contaminated rice oil with control children whose mothers were not exposed. Forty-four percent of children born to exposed mothers had abnormal middle ear morphology and/or impaired sound conductance compared with 18.8% of the controls. The identification of noninvasive methods of detecting thyroid system disruption of nervous systems end points such as impaired auditory thresholds may provide an important tool in laboratory and epidemiologic studies.

Summary

By superimposing the development of the thyroid system onto the chronology of brain development, it is evident that thyroid hormone may influence brain structure and function during the earliest stages of development in humans and rodents. Further investigation is needed to better understand the developmental patterns of thyroid-sensitive genes as well as their effects on brain development. Future versions of the timeline should also include development of the brain beyond birth and weaning, as many of the developmental processes continue well into adolescence in humans (48). In addition, species differences in the timing of brain development should be considered in the identification of thyroid-sensitive brain processes and developmental periods in mammals (136) and other vertebrates. Most important, as synthetic chemicals can interfere with nearly every step in thyroid system function, more research should be targeted

at understanding how thyroid-disruptive chemicals may impact normal brain development and functioning. By increasing the knowledge of thyroid hormone influence on brain development and how chemicals can disrupt thyroid system function, scientists can design more effective screens and assays to test chemicals for their possible impact on the thyroid system and downstream brain effects.

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